EFFECT OF FORAGE STAND TERMINATION METHOD AND FERTILIZATION HISTORY ON GREENHOUSE GAS EMISSIONS, NUTRIENT SUPPLY RATES, AND SOIL CARBON DYNAMICS

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ABSTRACT

While the majority of land used for growing forages in Saskatchewan is not fertilized on an annual basis, nitrogen (N) fertilization is often used to revitalize declining stands or for grass seed production. Once a stand is ready to be terminated, typically either a combination of tillage and herbicide or herbicide alone is used to kill the vegetation. Termination method is anticipated to have a significant effect on the rates and amounts of greenhouse gas (GHG) production, as well as affect carbon (C) and nutrient cycling in the soil. The objective of this thesis work was to examine the influence of grass forage stand termination method on GHG production, nutrient cycling, and dynamics of various soil C pools. Additionally, the influence of two previous years of N fertilizer addition versus no N fertilizer addition was examined. In a laboratory incubation of intact soil cores collected from two forage grass seed production fields in northeastern Saskatchewan (Arborfield brome grass in August 2013 and Carrot River timothy sites 1 and 2 in May 2014), termination by a combination of tillage and glyphosate caused a reduction of up to 16% in carbon dioxide (CO_2) emissions compared to glyphosate alone. The tillage/glyphosate termination also tended to decrease nitrous oxide (N_2O) emissions when compared to glyphosate alone. Prior N fertilization for two years resulted in increased emissions of both CO_2 and N_2O, as well as slightly lower phosphate (PO_4^{3-}) supply rates in the surface soil. Nitrogen supply rates were generally increased by N past fertilization, especially the ammonium (NH_4^+) supply rate, which was as much as 18% higher than in unfertilized plots. The field experiment conducted on the two Carrot River sites (CR1 and CR2) from August, 2013 to October, 2014 examined changes in soil organic C (SOC) pools. Prior N fertilization increased the amount of light fraction, water extractable, and microbial biomass C (LFOC, WEOC, and MBC, respectively) compared to the unfertilized plots. Termination with tillage significantly increased the LFOC concentrations in the following year but this difference disappeared by the end of the 2014 season. Tillage also tended to reduce the concentrations of WEOC and MBC over the course of the study. There were no significant differences between treatments in any of the C pools at the end of the study. Therefore, the conclusion of this thesis work is that the current practice of grass forage stand termination through a combination of tillage and glyphosate is a beneficial management practice in the soils studied through reduced greenhouse gas emissions.

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Dissolved organic carbon	DOC
Light fraction organic carbon	LFOC
Greenhouse gas	GHG
High-density polyethylene	HDPE
Microbial biomass C	MBC
Plant root simulator	PRS
Soil organic carbon	SOC
Soil organic matter	SOM
Total organic carbon	TOC
Urea Ammonium Nitrate	UAN
Water extractable organic carbon	WEOC

1. INTRODUCTION

Modification of the environment to better suit our needs is a practice that has been going on for thousands of years. Before the industrial revolution when the global population was under one billion people, land use change was never a significant problem. With over 7 billion people on the planet, land use change in response to population pressure is causing widespread environmental concern over land degradation and impacts on the global climate. The impacts of land use change on the cycling of carbon (C) and nitrogen (N) on local, national and global scales, especially carbon dioxide (CO_2) and nitrous oxide (N_2O), is an important consideration in soil quality and greenhouse gas budgeting (Hartmann et al., 2013).

A considerable amount of C is stored in soils across the globe, and management practices that change the size of this store have important implications for atmospheric CO_2 concentrations (Janzen et al., 1998). In most cases, the conversion of native prairie and forest to modern intensive agricultural cropping causes a decline in soil C for years after the conversion. As the demand for food continues to grow, more marginal land is being put into production. Kim and Kirschbaum (2015) estimated that land use change from natural forests to agricultural land contributed approximately 1175 Gt CO_2 equivalents over the time spanning from 1765 to 2005. A more recent estimate of mean annual global C emissions from land use change places that number at around 4.2 Gt CO_2 yr^{-1} between 1990 and 2009 (Houghton et al., 2012).

One potential means to increase soil C and move back towards pre-agricultural levels of soil organic carbon (SOC) is through the use of perennial forage crops. In east-central Saskatchewan, planting forage crops on annually cultivated or disturbed marginal land has been estimated to increase SOC content in the top 15 cm by 0.6 – 0.8 Mg C ha^{-1} yr^{-1} (Mensah et al., 2003). The C sequestration ability of forages can therefore be used as a tool to mitigate climate change, and with nearly 9 Mha of forage land in Saskatchewan alone, the mitigation potential is substantial. On top of improving soil quality, using forages in a crop rotation also reduces input costs, lowers financial risk, and extends crop rotations (Entz et al., 1995).

Currently the majority of forages in Saskatchewan are in the form of native rangeland (4.8 Mha). However, a significant amount is still actively managed, with 2.1 Mha of tame and seeded pasture, 1.5 Mha of alfalfa, 405 kha of tame hay, and 30 kha of land used for forage seed production which is the most extensively managed (Statistics Canada, 2011). Once established, most forage stands receive very little input, with significant fertilization occurring only in older stands and land used for forage seed production. In grass dominated forage stands, like the ones in this study, nitrogen (N) fertilizer is used to replace N that is removed with the crop and increase forage yield and protein content (Lkhagvasuren et al., 2011). The most common practice is to broadcast urea fertilizer in the fall, where fewer time constraints and drier soils make it more practical. The downside of broadcasting urea fertilizer without incorporation into the soil, whether mechanically or by rainfall, is that there is significant gaseous N loss through ammonia volatilization (Fenn and Hossner, 1985). Adding N fertilizer as urea ammonium nitrate (UAN) solution to grass dominated stands in Saskatchewan was shown to increase the availability of N and grass yield and protein in the season of application but did not increase available N supply rates, CO_2 or N_2O evolution in soil cores collected in the fall at the end of the season (Lkhagvasuren, 2007).

Ultimately in tame forage production systems, the stand is usually terminated after a few years due to declining production from weed infestation, low fertility and stand composition changes, and either re-seeded to forage or put into annual crop production. When a producer decides to remove the stand and return the field to annual cropping, stand termination is carried out one of three ways: tillage, herbicide, or a combination of the two. Tillage is generally viewed as having detrimental effects on soil properties such as water holding capacity, soil structure, and oxygen availability, all of which play a role in greenhouse gas (GHG) production and SOC dynamics (Dexter, 1997; Ogle et al., 2005). There are also fossil fuel emissions and energy costs associated with tillage operations. According to a survey of Saskatchewan and Manitoba forage producers, when using tillage alone, approximately five to seven passes across the field were needed to effectively terminate the forage stand and prepare a seedbed for the subsequent crop, which represents a significant input cost to the grower (Saskatchewan Agriculture, n.d.).

The alternative to tillage is chemical termination, which is typically done with a non-selective herbicide like glyphosate. A seeding tool which is capable of seeding directly into sod is used to seed the following annual crop. There have been many studies examining the potential

effects of glyphosate on soil microorganisms and their activity, and in general the reported effects are very small and short-lived (Carter et al., 2007). In studies looking specifically at effects on nitrification rates, they found no effect at concentrations that would be encountered in an agricultural setting (Stratton, 1990; Stratton and Stewart, 1991). Conversely, a study that looked at denitrification rates in a grass sward in Ontario found that glyphosate addition increased denitrification rates (Tenuta and Beauchamp, 1995). Termination of an alfalfa stand in northeastern Saskatchewan using herbicide was reported to slow the release of available N as ammonium (NH_4^+) and nitrate (NO_3^-) from mineralization as compared to termination by tillage (Malhi et al., 2010).

Information on GHG emissions, especially N_2O, from forage systems in the Northern Great Plains are extremely limited. Of particular note is that for grass stands, the effects of method of stand termination and N fertilization history on greenhouse gas emissions and soil C sequestration have not been investigated to date. The overall goals of this study are to determine how forage stand termination method and N fertilization history affects the emissions of N_2O and CO_2, and the forms and amounts of C in the soil following termination. It is hypothesized that termination of a grass stand by a combination of tillage and glyphosate herbicide will increase CO_2 emissions and reduce C stores in the soil compared to herbicide alone and that a history of N fertilization will increase N_2O emissions and increase C decomposition.

The major objectives of this thesis are to assess the impact of forage stand termination method and N fertilization history on GHG emissions and soil nutrient and C dynamics. The first two chapters serve to introduce the topic of the thesis and provide a review of the current literature. Chapters 3 and 4 contain the main body of research, with each covering a separate study to address a research question in this thesis. A laboratory incubation of intact soil cores was completed in Chapter 3 to observe the influence of fertilization history and stand termination method on N_2O and CO_2 emissions as well as nutrient supply and leaching rates. The fourth chapter examines changes in soil C fractions over the year following stand termination, measured by tracking concentrations of light fraction, water extractable, microbial biomass, and total organic C in the surface soil layer. The fifth chapter contains a synthesis of the results, final conclusions, and suggestions for future research. References cited across all chapters are listed in Chapter 6.

2. LITERATURE REVIEW

2.1 Forage Systems

The term forage refers to plants that are consumed by livestock and includes pasture and browse plants, hay, silage, grasses and legumes, alfalfa, immature cereals, and cereal straw. Forages play an important role in livestock operations in western Canada since they are usually a cheaper source of feed compared to grains. Due to the cold climate, hayed or stockpiled forages are a necessity for Canadian livestock operations for feed over the winter, and they typically meet the majority of livestock dietary requirements in cow-calf operations. Forage crops are either a single species or a mixture of legumes and grasses. In general, forages are typically grown on marginal land with low inherent fertility and their production can be significantly increased with fertilization (Lkhagvasuren et al., 2011).

The two main types of forages, legumes and grasses, differ in their nutrient composition and soil benefits. In general, legumes are high in protein while grasses are high in carbohydrates (Sengul, 2003). Legumes are beneficial due to their ability to enhance the N status of soil by fixing atmospheric N through symbioses with diazotrophs. Each year, about 20-22 Mt of N are fixed globally by cropped legumes compared to 85 Mt of N applied as fertilizer (Peoples et al., 2009). Conversely, grass forage crops have large, robust root systems (Fig 2.1) that improve soil structure, aeration, and water movement as well as increase soil organic matter.

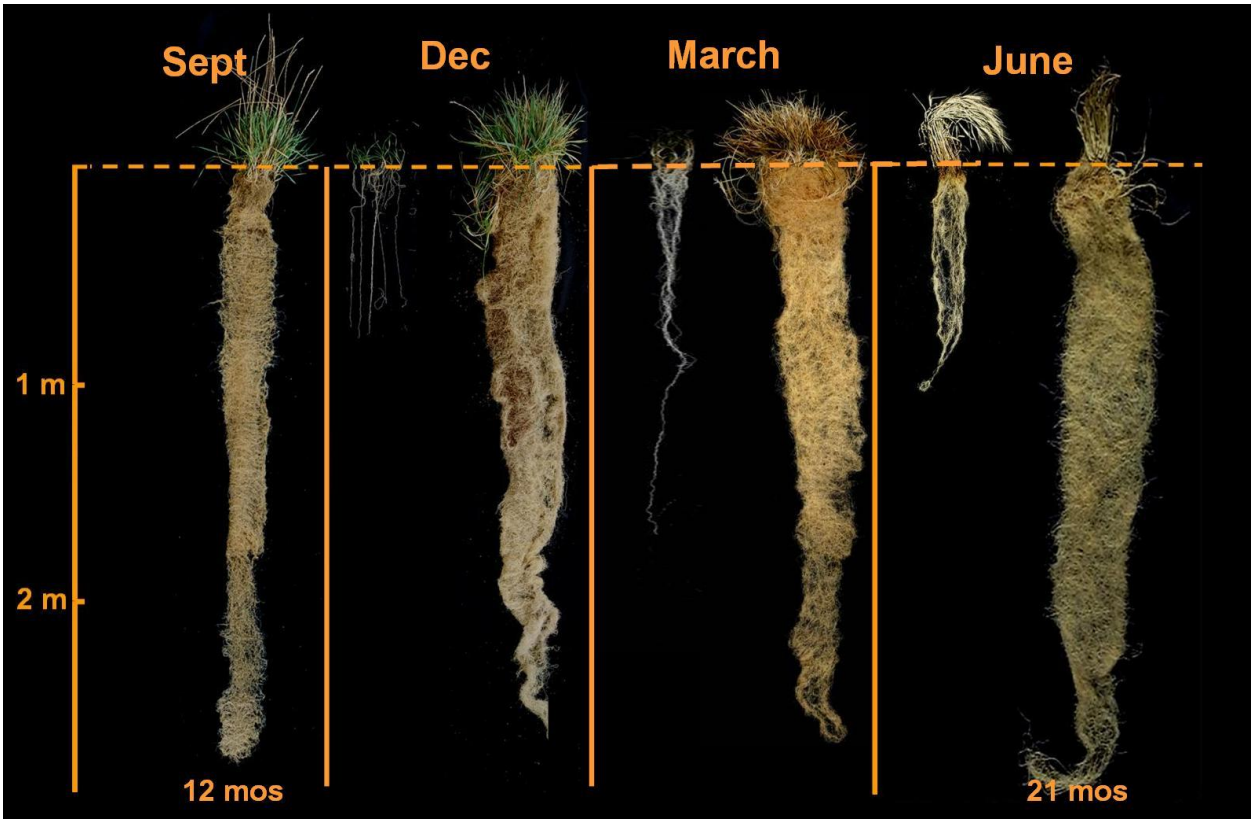

Figure 2.1 Image comparing root systems of an annual wheat (shown on left side of each column) to a perennial wheatgrass. Photo credit: Dehaan (Jerry Glover) Creative Commons via Wikimedia Commons.

In forage production systems of the Canadian prairies, the majority of stands are minimally managed once established. While the data is fairly limited on the rate of C sequestration, it has been estimated to be in the range 0.6 – 0.8 Mg C per hectare per year in east-central and north-eastern Saskatchewan (Mensah, 2003). When combined with the length of time forage stands remain planted, this represents a significant increase in soil C storage. The challenge comes in retaining that stored C when it comes time to terminate the stand. Typically, stands are terminated by either spraying with a non-selective herbicide like glyphosate, by cultivation, or a combination of both (Saskatchewan Forage Council, 1998a; 1998b).

2.1.1 Timothy grass [*Phleum pratense* (L.)]

Timothy is a cool-season perennial bunchgrass that is medium-lived, with a shallow, fibrous root system. Timothy grass is native to most of Europe. In Canada, it is used primarily in forage mixes for hay, pasture, or silage, but it is also used for seed production due to its high production of seeds, yielding up to 394 kg ha^{-1} in Manitoba and up to 560 kg ha^{-1} in the southern

United States (Entz et al., 1994; Lacefield et al., 2002). Manitoba is the main region producing timothy seed in Canada with seed production also occurring in the Peace River region of Alberta and in the northern agricultural region of Saskatchewan.

Proper residue management is important to maximize timothy seed production, as the amount of solar radiation that reaches the base of a timothy plant has a positive influence on tiller production and the subsequent conversion of vegetative tillers to reproductive tillers (Entz et al., 1994). According to Loeppky et al. (1999), timothy seed yield is also significantly influenced by N and P fertilization, with an average yield increase of 111% at a fertilization rate of 100 kg N ha^{-1} yr^{-1} and 32% increase with 18 kg P ha^{-1} yr^{-1} compared to unfertilized controls. This response to fertilizer was highly dependent on the initial nutrient status of the soil, with the yield response decreasing with increasing soil test N and P.

2.1.2 'Success' hybrid bromegrass [*Bromus riparius* Rehm. (L.) x *Bromus inermis* Leyss. (L.)]

'Success' is a hybrid bromegrass cultivar generated by crossing meadow bromegrass [*Bromus riparius* Rehm. (L.)] with smooth bromegrass [*Bromus inermis* Leyss. (L.)] and it was developed at the Saskatoon Research Centre of Agriculture and Agri-Food Canada in 2003. It is used in both hay and pasture systems, producing a high quality, high volume first cut hay crop, followed by fast regrowth for grazing (Peace River FOR-SiD, n.d.). It has short, slowly spreading rhizomes that result in the hybrid being less invasive than smooth bromegrass in pasture mixtures. The hybrid brome is reported to be best adapted to the drier, Brown soil zone areas of the prairies, producing more hay than both smooth and meadow bromegrass (Coulman, 2006), although it is grown throughout the prairie region. In a trial conducted at Saskatoon, 'Success' started regrowth faster after cutting and had higher dry matter yield than smooth bromegrass while also having similar or superior yields to meadow bromegrass and 'AC Knowles' hybrid bromegrass (Coulman, 2006). There have not been any studies specifically looking at the response of 'Success' dry matter or seed yields to fertilizer application. When comparing unfertilized grass monocultures of hybrid and smooth bromegrass to grass-alfalfa mixes, dry matter yields declined over time in response to decreasing soil N, while the grass-alfalfa mixes maintained dry matter yields over the study suggesting that hybrid bromegrass would be responsive to N fertilization (Foster et al., 2014).

2.1.3 N fertilization in forage systems

Since forages are typically grown on low fertility soils, yield increases due to N fertilization in forage systems has been well documented (Loeppky et al., 1999; Malhi and Gill, 2002; Sengul, 2003). As the stand ages, productivity tends to decline as a result of reduced stand vigour, invasion of weeds, and decreasing soil fertility. Typically, when this happens, the stand is either terminated and re-seeded to forage or is put into annual crop production (Kruger, 1997). Tillage is an energy intensive process that represents a significant input cost for the producer and it may be economically more viable to revitalize the stand by fertilization. Using N fertilizers to revitalize forages and increase yield production has been examined over many years (Ukrainetz et al., 1988; Fairey, 1991; Malhi et al., 2000, Kering et al., 2011). Nitrogen is typically the most common nutrient deficiency in soil and N fertilizer generally has the greatest impact on production of forages (Malhi et al., 2004), especially on grasses like bromegrass (Lkhagvasuren et al., 2011).

Available soil N and seasonal moisture are major controls on the effect of N fertilizer on forage seed yields. A study by Loeppky et al. (1999) examining forage seed yield response to N and P fertilizers showed that both moisture and soil N level had a significant effect on the yield response of bromegrass. During the course of the study, in the driest year the seed yield was only 0.29 t ha^{-1} whereas in the wet year the seed yield was 1.24 t ha^{-1}. Comparing plots with equal soil test P, plots with higher soil test N produced 36 – 73% higher seed yields.

2.2 Forage Stand Termination

2.2.1 Tillage

Tillage is defined as the mechanical manipulation of soil to enhance crop growth. Tillage is generally viewed as an essential part of agricultural management, but inappropriate tillage practices and over tilling can have detrimental effects on the sustainability of land resources through soil erosion and soil organic matter loss (Abdalla et al., 2013).

Prior to the discovery and production of herbicides, tillage was the only means to reduce populations of undesirable plants (weeds) as well as stimulate nutrient cycling. Indeed, tillage is an effective form of weed control. A study by Zarzecka et al. (2009) showed that plough tillage significantly reduced weed populations in potato crops compared to reduced tillage. Tillage is successfully employed in organic agriculture where use of chemical herbicides is prohibited

(Johnson et al., 2012; Stepanovic et al., 2015). Tillage is a significant driver of soil organic matter turnover by incorporating and redistributing the organic matter throughout the topsoil, disrupting soil aggregates and increasing aeration, which produces conditions suitable for mineralization (Peigne et al., 2007). This in turn affects CO_2 emissions and soil C stores.

Disruption of soil aggregates through tillage exposes organic matter inside the aggregates to increased oxygen and increases the surface area available to decomposing micro, meso and macro fauna, increasing respiration. Carbon dioxide fluxes have been shown to be strongly influenced by soil temperature (Eriksen and Jensen, 2001), and tillage has been shown to increase soil temperature compared to no-till systems (Sainju et al., 2012), especially in the early spring. The combined effects of tillage on soil temperature, aeration and surface area susceptible to decomposition helps explain why CO_2 emissions are typically higher in cultivated systems versus no-till systems (Eriksen and Jensen, 2001; West and Marland, 2002; Boeckx et al., 2011; Sainju et al., 2012). The increased CO_2 emissions associated with conventionally tilled systems are also suspected to be partly due to the physical release of CO_2 from soil pores and solution (Eriksen and Jensen, 2001).

Tillage contributes to soil erosion through breaking up aggregates and reducing soil moisture as well as through physical relocation of the soil. Historically, research has focused on the influences of water erosion on soil removal but since the widespread application of Caesium isotope techniques, evidence of tillage induced soil translocation has been documented. Xiaojun et al. (2013) reports that while water erosion is the major process of soil redistribution in locations with gentle slopes, tillage erosion is the dominant process of soil redistribution in steeply sloping topography. It appears that tillage erosion is closely tied to tillage depth, as animal powered farming systems in Cuba, where tillage depth is under 4 cm, are reporting significantly lower erosion rates (Wildemeersch et al., 2014).

In light of these detrimental effects of soil tillage, conservation tillage, in the form of reduced or no-till management has been gaining popularity in conventional agriculture. Reduced tillage aims to decrease the number of passes or tillage depth, while no-till eliminates it entirely and uses equipment that minimizes soil disturbance. In the no-till system, control of undesired vegetation is accomplished through herbicide application. Where conventional tillage practices typically do not leave crop residues on the soil surface, conservation tillage maintains a minimum of 30% on the soil surface (Peigne et al., 2007). The crop residues left over help

conserve soil, organic matter, water, and generally increase crop production (Malhi et al., 2006). In a no-till cropping system that includes forages in rotation, the forage stand is terminated with herbicide rather than tillage.

Termination of a forage stand can be accomplished by tillage alone but five to seven passes are generally required to completely remove the stand (Hall, 2016). While intensive tillage has a secondary benefit of leveling out the ground, it contributes to soil erosion and degradation, loss of soil moisture, as well as having high time, fuel, and equipment costs. In Saskatchewan, tillage is typically carried out with either a plough or a disc (Figure 2.2). A plough is used for deep tillage, where the soil, crop residue, and root mass are pulverized and inverted, causing significant mixing. A disc is typically a shallower tillage operation but covers a larger area. Rather than using chisels like in ploughs, a disc uses concave discs to dig up and mix the soil and crop residues. Tillage operations are generally carried out in the fall to avoid losing out on a year or partial year of production. This is also when the plant energy status is relatively low which reduces the energy required to break up the soil and crop residue (Nybo, 2015). Spring cultivation is used less often due to high moisture and time constraints.

Figure 2.2 Mouldboard plough (Massey Ferguson, 2018) and tandem disc (Versatile, 2018) tillage implements.

2.2.2 Chemical termination

Glyphosate is a systemic herbicide that is useful for terminating the growth of annual and perennial plants including grasses, sedges, broad-leaved and woody plants. There has been speculation that glyphosate negatively affects the soil microbial biomass C (MBC), but studies have shown it to be either inconsequential or to positively increase microbial biomass (Wardle and Parkinson, 1991; Busse et al., 2001; Carter et al., 2007; Mijangos et al., 2009). This effect is

most likely due to glyphosate acting as a C source for microbial growth. The effects on soil respiration and CO_2 emissions when a forage stand is terminated with glyphosate compared to tillage are likely to be influenced by shoot and root mortality, soil oxygen and water status, and availability of other nutrients. However, information on effects of termination methods is scarce.

The reported impacts of glyphosate on the N cycle and N_2O production in soil are also mixed. A study by Stratton (1990) using soils from Truro, Nova Scotia aimed to quantify the effect that glyphosate has on nitrification rates in soil at normal field exposure rates. The nitrification rate in the near neutral (pH 6.8) sandy loam soil was stimulated by glyphosate addition but not until glyphosate concentrations reached 50 times normal exposure rates. The most acidic soil (pH 5.8) was inhibited at only 10 times the normal exposure rate suggesting that the inhibition effect increases with increasing acidity. When looking at the effect of glyphosate on denitrification rates, Tenuta and Beauchamp (1995) found that denitrification rates increased following application, attributing this to increased NO_3^- and moisture availability from the death of vegetation and removal of plant N sinks.

Glyphosate use is very widespread in Saskatchewan agriculture as a substitute or supplement for traditional tillage. Herbicide spraying is faster and cheaper than tillage for stand termination but the timing is more important and heavier duty equipment is needed for seeding. The choice between tillage and herbicide use depends on individual environmental factors such as moisture and salinity. Moisture and salinity problems are exacerbated by intensive tillage so glyphosate is recommended in these areas (Nybo, 2015). On the other hand, no-till systems require special seeding tools such as a disc or narrow knife seeder to adequately prepare the seed bed for the following crop.

Spraying is recommended in late summer to fall as it requires an actively growing plant for maximum effectiveness (Hall, 2016). Waiting for regrowth in the spring for spraying delays seeding dates by 2-3 weeks and is generally not recommended. Temperature is another important factor for timing recommendations as glyphosate is most effective between 16-24 °C (Roundup, 2015). Typical rates of glyphosate application in Saskatchewan are 1.6 - 3.3 L ha^{-1}.

2.2.3 Burning

Burning may be conducted as a low-cost part of the termination process to reduce crop residues after harvest. In years with significant crop growth or late season moisture, spreading

and incorporating crop residue can be difficult. Additionally, burning is used for removing tough residues like flax or where soils are prone to compaction. While nearly all of the C, N, and sulfur (S) are lost from the residues, mineral elements like P and potassium (K) remain in the soil (Gelderman, 2009).

Burning releases particulate matter, CO_2, N oxides, S dioxides, carbon monoxide, methane, and other volatile organic compounds (Kanabkaew and Oanh, 2010). While burning does not appear to have a large effect on total C in a soil in the short-term (Wuest et al., 2005), it has been shown to increase the activity of the urease enzyme, which may have consequences for emissions from urea application (Ajwa et al., 1999). The effect of burning on increasing activity of urease enzyme, responsible for hydrolysis of urea to ammonia gas, appears to only develop after many seasons of burning, as no effect was detected within the first year after burning (Picone et al., 2003).

Removing plant residues by burning can have other effects on soil quality and productivity. Podgaiski et al. (2014) reported that burning plant residues in a grassland reduced soil fauna densities and surface feeding activities of detritivores for up to 6 months following burning, attributing it to mortality caused directly by heating. Burning also has an effect on C and N cycling in soils by increasing recalcitrant plant inputs to SOM. Using radioisotope labeling, Soong et al. (2015) found that pyrogenic organic matter added to SOM is largely untransformed, and in N limited sites, litter N was tightly conserved. The effect on soil N transformations from burning was examined by measuring N_2O emissions from differing management regimes. In a study in Queensland, Australia, fertilized plots that had wheat residues removed by burning had emissions of N_2O that were 259g N ha^{-1} yr^{-1} less than plots with the residues retained (Wang et al., 2011).

2.3 Agricultural Greenhouse Gas Production

Agriculture is reported to account for approximately 8% of Canada's total emissions of greenhouse gases in 2013 on a CO_2 equivalent (CO_2 eq) basis (Environment Canada, 2015a). Nitrous oxide emissions from Canadian agriculture are disproportionately high and account for 72% of Canada's total N_2O emissions (Environment Canada, 2015a).

2.3.1 Carbon dioxide

Carbon dioxide is the main greenhouse gas and comes from many different sources, accounting for about 80% of Canada's total CO_2 eq greenhouse gas emissions (Environment Canada, 2015a). The contribution of CO_2 emissions from agriculture is relatively small. When compared to N_2O and methane on a CO_2 equivalent basis, CO_2 accounts for just over 20% of agricultural emissions.

Respiration by soil micro, meso and macrofauna as they decompose organic materials is an important part of the global C cycle. Soil respiration rates can be altered by global scale changes such as atmospheric composition that affects soil temperature and moisture, N deposition and land-use management practices (Jia et al., 2012). Greater understanding of soil responses to management practices is needed to improve estimations of terrestrial C balances.

It is well known that tillage of cropland generally increases CO_2 emissions. Several studies have shown that tillage increases soil CO_2 emissions compared to zero-till or reduced tillage (Suave, 2000; West and Marland, 2002) with the largest increase in emissions coming from cultivating land that has remained uncultivated for many years (Shahidi et al., 2014). Soil CO_2 flux is controlled by several plant, soil, and climatic conditions that are affected by tillage. Age, amount, and the C:N ratio of plants affect respiration rates (Parr and Papendick, 1978; Ghidey and Alberts, 1993; Finn et al., 2015). Microbial respiration is also controlled by soil temperature, water content, oxygen availability, and available nutrients (Reicosky et al., 1997; Phillips and Podrebarac, 2009; Fernandez et al., 2014).

Urea fertilizer is known to affect CO_2 emissions from soil. When urea is applied to soil, in the presence of water and urease enzyme the urea is converted to NH_4^+, hydroxyl ions, and bicarbonate. The bicarbonate ions then react with hydrogen to form water and CO_2, evolving the CO_2 that was fixed in the industrial production process (Snyder et al., 2009). Secondly, urea can affect CO_2 emissions via possible stimulation of microbial C cycling. For example, in a study by Phillips and Podrebarac (2009) in North Dakota, they found that urea fertilization increased net fluxes of CO_2 in both cultivated and native prairie soils in North Dakota.

2.3.2 Nitrous oxide

Nitrous oxide is a potent GHG with a 100-yr global warming potential of approximately 310 times that of CO_2 (IPCC, 2007b). On average, the residence time of N_2O in the atmosphere

is about 120 years before being removed by a sink or destroyed through chemical reactions such as the destruction of stratospheric ozone (Environmental Protection Agency, 2014). As of 2010, the total annual global emissions of N_2O were about 9 million metric tons, which equates to an average yearly increase in atmospheric concentration of 0.8 parts per billion (IPCC, 2007a; The World Bank, 2014).

In Canada, agriculture is the single greatest emitter of N_2O, accounting for 72% of the total N_2O emissions (Environment Canada, 2015a). Nitrous oxide emitted from the soil can be a by-product of nitrification, denitrification, or by a combination of both (Khalil et al., 2004). Emissions of N_2O are highly variable and are influenced by site-specific conditions, particularly the availability of water and oxygen, and application of inorganic N. Nitrous oxide is produced from microbial transformations of inorganic N, and potential emissions increase with increasing N availability (Bouwman et al., 1993). Since it is common practice to apply inorganic fertilizer to many agricultural fields, the potential for N_2O emissions is high.

The high variability of N_2O emissions poses a problem when attempting to provide accurate estimates of emissions from a landscape. The simpler method for estimating emissions is to remove intact soil cores from a field, place them in sealed incubation chambers, and measure GHG flux over an hour each week (Nelson et al., 2007). Cumulative gas production is estimated by interpolating between data points and integrating over time assuming a constant flux. Since there is high temporal variability of N_2O emissions (Yates et al., 2006), this method tends to underestimate total emissions (Hensen et al., 2013) and, while effective in reducing variability and revealing treatment effects, the magnitude of the emissions likely does not reflect the field due to differences in temperature and moisture. Part of the reason this method tends to underestimate total emissions is probably due to most incubations being done at water filled pore space levels below that where significant N_2O production occurs in the field.

Nitrous oxide is produced through two main pathways: nitrification and denitrification. Nitrification is the conversion of NH_4^+ to NO_3^- and is a two-step process. In the first step, *Nitrosomonas* and *Nitrosopira* species oxidize NH_4^+ to nitrite (NO_2^-). This NO_2^- is further oxidized into NO_3^- by *Nitrobacter* species. Nitrous oxide can be produced during this process when NO_2^- is used as an alternate electron acceptor during NH_4^+ oxidation, particularly when O_2 pressure is low (Khalil et al., 2004).

Nitrous oxide is also produced through denitrification, where facultative anaerobic microbes reduce NO_2^- or NO_3^- into gaseous nitric oxide (NO), N_2O, or N_2 (Hutchinson, 1995). The main factor controlling denitrification is the presence of O_2, which limits the activity and synthesis of denitrifying enzymes in soil (Khalil et al., 2004). When O_2 pressure is low, denitrifiers utilize NO_2^- and NO_3^- as an electron acceptor. Denitrification enzymes can remain in the soil for several months under aerobic conditions, becoming active once the soils are rewetted and saturated (Nelson, 2003).

There are many factors influencing nitrification and denitrification rates, mainly soil moisture, soil N status, temperature, and soil texture. Soil moisture affects the ratio of water-air filled pore space. Since nitrification is an aerobic process, water filled pore space above 60% hinders the nitrification process (Hutchinson, 1995). Soil N status determines the amount of substrate available for both processes, and low N supply rates generally mean lower nitrification and denitrification rates (Nelson, 2003). Soil temperature is a significant driver of N_2O production. At soil temperatures <4 °C, nitrification is severely limited (Ma, 2009). Nitrous oxide emissions from nitrification also increases exponentially with temperatures between 15-35 °C (Nelson, 2003). Temperature both directly and indirectly affects rates of denitrification through controlling the activity of denitrifiers, increasing O_2 consumption by heterotrophic microbial activity, and by influencing the solubility and diffusion of O_2 (Ma, 2009).

The effect of fertilization on N_2O emissions is rather evident in the literature. In general, as more fertilizer N is applied, the N_2O emissions increase. This has been verified in a large number of studies examining GHG emissions and varied rates of fertilizer in the laboratory and field (Baggs et al., 2003; Malhi et al., 2006; Malhi and Lemke, 2007; Liu et al., 2011; Pelster et al., 2011; Sainju et al., 2012; Hangs et al., 2013). On the other hand, the effect of tillage on N_2O emissions is not as clear. Some studies have shown that N_2O emissions increase with reduced or no-till (MacKenzie et al., 1997; Baggs et al., 2003; Venterea et al., 2005; D'Haene et al., 2008; Abdalla et al., 2013), while others have shown the opposite, mixed, or no effect (Malhi et al., 2006; Malhi and Lemke, 2007; Boeckx et al., 2011; Pelster et al., 2011; Sainju et al., 2012). The many interacting factors affecting N_2O emissions makes it rather difficult to predict the effect that tillage will have, as tillage affects O_2 availability, soil pores, and soil temperature.

2.4 Soil Carbon Fractions

In the soil, the concentration of soil organic carbon (SOC), water extractable organic carbon (WEOC), light fraction organic carbon (LFOC), and MBC can all be measured. Of interest in the soil C cycle are the relative amounts of the different operationally defined fractions and changes that occur as a function of treatment, as this can be used to predict rates of C turnover and as a sort of proxy for soil health (Liang et al., 2003; Corvasce et al., 2006; Mohammadi et al., 2012). The response of these fractions to management practices including tillage and fertilization are of interest and considerably varied (Song et al., 2014).

2.4.1 Soil organic carbon

While total SOC will likely have the lowest percentage change in the short term and be least sensitive to treatment effects, it is useful for quantifying the total rate of C sequestration in a soil. Perennial forages in a rotation can be an important component of sustainable agriculture by increasing soil organic matter (SOM) levels through biomass contributions (Norton et al., 2012). However, there appears to be a dichotomy between above- and belowground biomass production from forages, where management approaches that increase aboveground biomass simultaneously decrease belowground biomass (Ghimire et al., 2014). Limited N supply in soil results in greater belowground biomass production to access more mineral N, but the N limitation stunts aboveground growth (Anderson, 1988; Monks et al., 2012). Soils under reduced and no-till management tend to make root mass concentrate near the surface due to increased moisture and nutrient availability (Beegle, 1996).

In forage production systems, SOM is increased through minimal soil disturbance and increasing root biomass compared to annual cropping systems (Paustian et al., 1997). Increase of SOC in systems where aboveground biomass is removed is primarily through roots and rhizodeposition (Van der Krift and Berendse, 2002) where roots constitute up to 30% of total SOM (Stevenson and Cole, 1999). The higher SOM in reduced tillage management systems is likely a result of greater root biomass accumulation as well as slower rates of root turnover (Ghimire et al., 2014).

2.4.2 Water extractable organic carbon

Water extractable organic carbon represents the most active and mobile form of organic matter in soil. Solubility renders the WEOC readily available for decomposition and also to move long distances with water moving in the soil profile. It is an operationally defined pool of organic C and is typically defined as organic matter smaller than 0.45 µm, with larger particles being labeled as particulate organic matter (Corvasce et al., 2006). Generally, dissolved organic matter is used to refer to any organic matter that is truly dissolved in situ, with WEOC being used to refer to the proportion of dissolved organic matter that can be gently extracted with a 5 millimolar calcium chloride solution in mineral soils or distilled water in organic soils (Chantigny et al., 2008). Water extractable organic carbon is generally used as a surrogate for dissolved organic C but may also include some organic matter that is released through physical disruption of the soil structure as well as desorption from exchange sites (Zsolnay, 2003).

Land management practices such as tillage and N fertilization can alter physical and chemical properties of the soil that are likely to influence WEOC dynamics. Incubation studies have shown that up to 44% of WEOC is degradable by microorganisms and contains both rapidly and slowly degradable fractions (Sun et al., 2015). In minimal and zero tillage regimes in arable soils, WEOC is found at much greater concentrations at the soil surface compared to conventional or deep plough practices (Sun et al., 2011).

Water extractable organic carbon is increased in the presence of grasses. Some WEOC originates from aboveground plant litter and throughfall, but the majority comes from root turnover and rhizodeposition (Nguyen, 2003; Schwendenmann and Veldkamp, 2005). While the presence of grasses significantly increases the quantity and cycling of WEOC, the individual species of grass appears to make little difference (Khalid et al., 2007).

2.4.3 Light fraction organic carbon

The light fraction of organic carbon is operationally defined as the proportion of organic matter that floats in a solution with a specific gravity ranging from 1.6-2.0 (Sollins et al., 1999). It encompasses organic residues that are partly decomposed and thereby relatively recent and which represent a large proportion of the substrate available for soil microorganisms (Larney et al., 1997). Typically, a sodium iodide solution with a specific gravity of 1.7 is used because it

separates most organomineral and mineral particles from decaying plant residues (Gregorich and Beare, 2008).

Nitrogen fertilization of grass stands typically increases the amount of LFOC in soil. A study done by Malhi et al. (2003) found that LFOC increased with increasing rate of N fertilizer, likely due to the increased plant biomass production that comes from fertilizer application. A similar outcome was found in Alberta, where N input coupled with less frequent fallow increased the amount of LFOC in the soil (Smith et al., 2015). Application of liquid manures was also shown to increase LFOC in soil, especially in soil of higher clay content (King et al., 2015).

Light fraction organic carbon has also been shown to increase when utilizing minimal or no tillage, but it takes several years to result in significant detectable differences. A study by Soon et al. (2007) showed a significant increase in soil LFOC content from no-till compared to conventional till after the 8th year of the study. An identical outcome was reported by Larney et al. (1997), where no-till increased LFOC content by 15-27% compared to conventional tillage, but also not significant until after 8 years. Liang et al. (2003) found that tillage had very little effect on LFOC except in the Black soil zone, where no-till had higher amounts of LFOC as well as a higher LFOC:SOC ratio.

2.4.4 Microbial biomass carbon

Microbial biomass consists mostly of bacteria and fungi and is a measure of the living component of soil organic matter. The microbial community is responsible for the decomposition of plant and animal residues, which returns a portion of the C and nutrients to the soil to be used for plant uptake. Accumulation of microbial biomass primarily occurs in the surface layers of soil in no-till systems (Helgason et al., 2009), although this is not always the case (Drijber et al., 2000). Microbial biomass has been shown to vary greatly between conventionally tilled and untilled soil, with total microbial biomass typically being greatest in no till systems (Franzluebbers et al., 1994). Microbial biomass also has high variation over the course of a season, although part of this variation might be due to insufficient replications of the chloroform fumigation-extraction method (Joergensen et al., 1994; Patra et al., 1990).

In general, fertilizer application tends to increase microbial biomass in the soil. Lupwayi et al. (2010) reported that fertilizer application significantly increased microbial biomass in all but three cases, and attributed it to increased soil nutrients, root exudates, and crop residues. A

study by Mohammadi et al. (2012) also concluded that fertilizer addition increased microbial biomass, but that manure and compost had larger effects than synthetic fertilizer alone, likely due to increased biogenic materials including dissolved organic C and carbohydrates.

2.5 Soil Nutrient Cycling and Export

2.5.1 Nutrient supply rate

The flux of nutrient ion to an adsorbing surface in the soil is termed the nutrient supply rate and can be measured by using ion exchange resins that act as an ion sink for the duration that the resin is in contact with the soil (Qian and Schoenau, 2002). The resins come in many different shapes and sizes, but the most convenient form places a small sheet of resin in a plastic frame, resulting in an easily installable probe. The shape of the probe also makes it much easier to calculate surface area compared to traditional resin balls. There are two different exchange resins used, one for anions and the other for cations. Cation exchange resins are strongly acidic, using sulfonic acid functional groups, while the anion exchange resins are strongly basic, using tertiary NH_4^+ functional groups (Qian et al., 2008).

Traditional methods to measure nutrient supply rates involved incubating soil for more than 20 weeks at optimal conditions to assess potentially mineralizable N and its mineralization rate constant (Qian and Schoenau, 2005). The resin membrane probes can be placed in situ, adsorbing various ions in the soil. By replacing probes over time in the same soil slot, the rate at which nutrients may be supplied to plant roots can be elucidated and a total cumulative supply determined for the time period of interest.

Ion exchange resins can be used to measure short-term nutrient supply rates in soil, and prior research has shown good agreement between measured supply rates and plant uptake (Qian and Schoenau, 2005). Ion exchange resins have also been shown to be good tools to measure N mineralization rates, but the interaction of the resins with the soil can influence mineralization rates (Friedel et al., 2000).

2.5.2 Nutrient leaching

Nutrient leaching from agricultural land is a problem largely associated with fertilizer or manure over-application. The increase in loading and concentration of P in Lake Winnipeg has caused algal blooms to nearly double since the mid-1990s, largely because of rapidly increasing livestock production and synthetic fertilizer use (Schindler et al., 2012). Chesapeake Bay is also

experiencing eutrophication from high nutrient loading due to agriculture in the area, which has prompted regulatory action (EPA, 2018).

The increase in N leaching due to elevated fertilizer N use is well documented (Kopacek et al., 2013). The excess N in the system remains mobile especially when added as, or converted to, NO_3^- and is lost following rainfall events. A study examining the effect of tillage and urea application on P and N leaching rates found that while tillage had no effect on P leaching, it did have a significant inhibitory effect on N leaching, attributed to the destruction of macropore pathways (Han et al., 2015). Similarly, Cui et al. (2013) found that tillage tended to reduce NO_3^- and NH_4^+ concentrations in leachate water, but on some occasions significantly increased them. It appears that the better soil structure in no-till systems increases water movement and downward percolation which exacerbates N leaching.

3.0 Influence of Grass Forage Stand Termination Method and Nitrogen Fertilization History on Greenhouse Gas Emissions, Nutrient Supply Rates, and Nutrient Leaching Rates in a Laboratory Incubation

3.1 Introduction

Global interest in GHG emissions has been steadily increasing over the past century. According to the most recent National Inventory Report (Environment Canada, 2018), agricultural activities in Canada contribute approximately 8% (60 Mt) of the total GHG emissions on a CO_2 equivalent (CO_2 eq.) basis. Within the agriculture sector, just under half of the total emissions come from the soil (24 Mt CO_2 eq.) (Environment Canada, 2018). It is therefore imperative that we have a solid understanding of GHG sources and sinks so we can identify avenues to mitigate climate change.

Carbon dioxide is the baseline that is used to compare all other GHGs to because it is the most ubiquitous GHG. Atmospheric concentrations of CO_2 have increased every year since measurements began in the 20th century, rising from 316 ppm in the late 1950s to over 398 ppm in 2014 (NOAA, 2016). On top of the annual growth, the decadal mean growth rate is also increasing, due to increasing global population and industrialization. Carbon dioxide in agriculture is a by-product of the breakdown of SOM and increasing levels of organic matter in the soil tends to be associated with increased CO_2 emissions (Fernandez et al., 2014).

Nitrous oxide has a global warming potential approximately 310 times that of CO_2 on a 100-yr scale (IPCC, 2007b). Nitrous oxide is produced from many different activities, including agriculture, combustion of fossil fuels, industrial processes, and manure storage and application. In Canada, just under three quarters of the total N_2O emissions are attributed to the agriculture sector, with a similar situation in the United States (Environmental Protection Agency, 2014). Due to the high variability of N_2O emissions, it is difficult to accurately scale up spatially and temporally.

In agricultural systems, N_2O is a by-product of the N cycle, specifically nitrification and denitrification processes. Nitrous oxide production is dictated by environmental factors and

management practices that affect these factors. Oxygen availability is the largest factor controlling N_2O emissions, but soil moisture, soil N status, soil texture, and temperature also play a significant role. Increases in soil moisture, N availability, and temperature all tend to increase N_2O emissions. Disruptive management practices like tillage break up soil structure which affects O_2 status, soil pores, and soil temperature.

Generally, tillage of agricultural soils has been acknowledged to increase CO_2 emissions due to physically disrupting the soil aggregates, which by increasing aeration and access of decomposing organisms to the organic matter within, accelerates decomposition and CO_2 production (Abdalla et al., 2013). The effect of tillage on N_2O emissions is much less clear. Some studies have shown tillage to decrease N_2O emissions (Chatskikh and Olesen, 2007; Gregorich et al., 2008), while others have shown an increase (Ball et al., 1999; Rochette et al., 2008). Soil texture is a significant factor influencing N_2O emissions, contributing to variable effects observed among soils as it affects O_2 status, water availability, predominance of micro versus macro pores, and aggregation. Physical disruption of the soil through tillage affects all of these factors by destroying soil pores and aggregates, which in turn affects O_2 status and water availability.

Nitrogen fertilization on the other hand has been shown to have a rather significant consistent effect on N_2O emissions by providing substrate to the nitrifiers and denitrifiers. The effect of N fertilization on N_2O emissions is relatively short lived as once the substrate runs out production ceases. A global meta-analysis by Shcherbak et al. (2014) found that N_2O emissions exponentially increase as N inputs increase to eventually exceed crop uptake. Fertilizer application has also been shown to increase CO_2 emissions, although the effect becomes less consistent at high application levels (Xiao et al., 2005; Tanveer et al., 2013).

Forage systems in western Canada have relatively low GHG emissions when compared to typical annual crops (Maas et al., 2013). Once a forage stand is established on the prairies they typically receive very little chemical or mechanical input over the following years other than hay harvest. The exception is grass stands that are used for forage seed production, which are fertilized and harvested every year. The largest soil disturbance occurs when the stand is terminated and the field is returned to annual cropping. Due to the effects of tillage on the controls of GHG production, and the known effects of N fertilization, there is a need to

determine how stand termination method and fertilization history affect GHG emissions following termination.

The objectives of the study in this chapter were two-fold: 1) to compare N_2O and CO_2 emissions from forage grass stands terminated by a combination of tillage and herbicide versus termination by herbicide alone; and 2) assess the influence of a history of N fertilization compared to no fertilization of forage grass on greenhouse gas emissions. It is hypothesized that plots terminated by a combination of tillage and herbicide will have higher GHG emissions compared to herbicide alone. It is also hypothesized that a history of N fertilization will increase GHG emissions.

3.2 Materials and Methods

3.2.1 Site characteristics

Intact soil cores used for this experiment were taken from three sets of plots across two separate research fields. The first field (Fig. 3.1) is near Arborfield, SK (NE-12-48-11-W2; 13U, 602430 E, 5888005 N), and the other (Fig. 3.2) is approximately 10 km southeast of Carrot River, SK (SW-11-49-11-W2; 13U, 599694 E, 5896594 N). The Arborfield site is mostly level and is located on the edge of the boreal forest in the Dark Gray soil zone. Soils in the area are mainly a mixture of Tisdale association Orthic Dark Gray and Gleyed Dark Gray Chernozems on mid to lower slopes, with Arborfield association Solonetzic Dark Gray Chernozems on some lower slopes and in some depressions, and a mixture of Melfort association Orthic Black and Gleyed Black Chernozems on upper slopes and knolls (CanSIS soil survey, 1997b). The texture of the site is a clay loam. Timothy [*Phleum pretense* (L.)], variety ‘Comer’, was planted in 2009 and was terminated at the end of 2013. At the end of each growing season, the seed was harvested and the residue was baled.

Similar to the Arborfield site, the Carrot River sites are located on the edge of the boreal forest in the Dark Gray soil zone. The first site (CR1) is located along the northern side of the field (13U, 599783 E, 5896807 N) and the second site (CR2) is located in the southwestern corner of the field (13U, 599360 E, 5896275 N). The difference between the two Carrot River sites is mainly the texture, with CR1 being a sandy clay loam and CR2 being a sandy loam, but there is also a slight difference in pH and EC. The soils at CR1 have an average pH of 7.8 and an EC of 0.77, while the soils at CR2 are 7.7 and 0.53 respectively. The CR1 site also tends to collect water

right after spring thaw and spends a few weeks waterlogged at the start of the season. Soils in the area are mainly a mixture of Gronlid association Gleyed Rego Dark Gray and Gleyed Calcareous Dark Gray, with a mixture of Carrot River association Gleyed Dark Gray and Gleyed Calcareous Dark Gray soils on upper slopes and Gronlid association Gleyed Dark Gray soils on lower slopes. The topography is mostly level with a slight slope (<1%) extending downward to the northeast corner or the field. A major difference between the Arborfield site soil and the Carrot River sites soil is the texture (clay loam at ABR, sandy clay loam at CR1, and sandy loam at CR2).

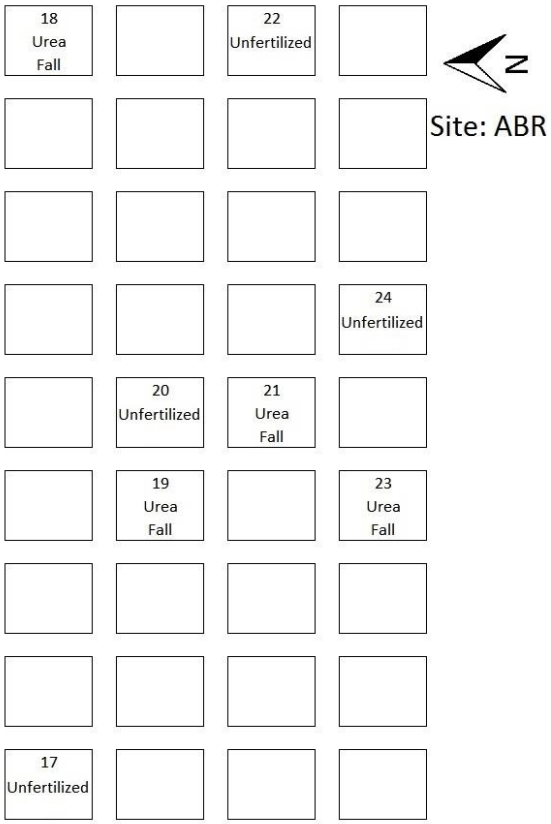

Figure 3.1 Study plot diagram showing sampled plots at the Arborfield site from which cores were taken and used for the research in this thesis. Each column represents one block of replicates. Plot ID number and fertilization history is only included for plots used in this study.

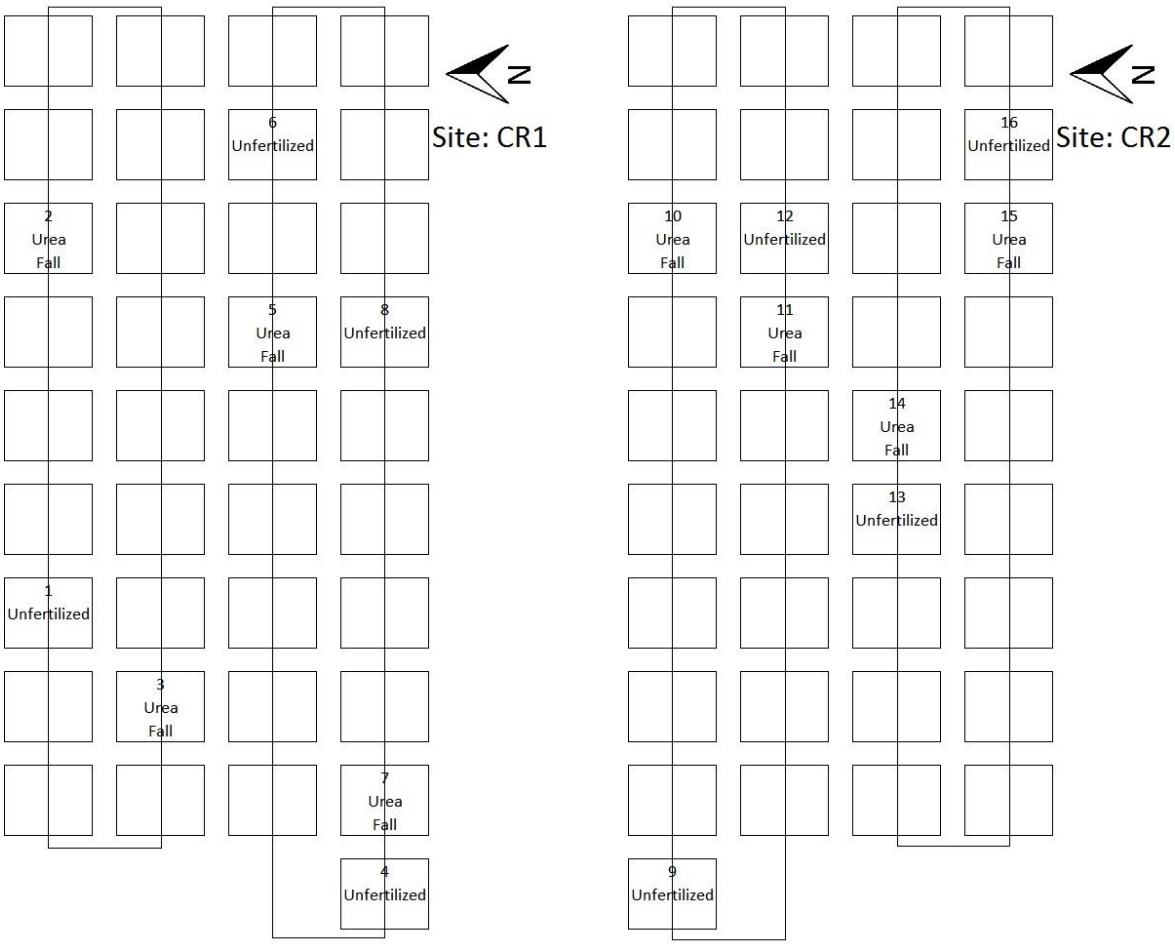

Figure 3.2 Study plot diagram showing sampled plots at the two Carrot River sites from which cores were taken and used for the research in this thesis. Each column represents one block of replicates (plot 4 was relocated from block 2 due to cooperator operations). Vertical rectangular boxes denote area that was cultivated by tandem disc for tillage termination treatments. Plot ID number and fertilization history is only included for plots used in this study. Plots 4 and 9 were relocated due to drainage ditch construction and small-scale N fertilizer application, respectively.

The field was seeded with hybrid bromegrass [*Bromus inermis* Leyss. (L.) *x Bromus riparius* Rehm. (L.)], variety 'Success', in 2010 and was grown for 4 years, with the stand being terminated at the end of 2013. At the end of each growing season, the seed was harvested, the residue was baled, and the remaining stubble was burned at the beginning of the following season.

Due to their proximity (<10 km), the Arborfield and Carrot River sites have identical climates. The region has a mean annual temperature of 1.4 °C and mean annual precipitation of

447 mm, with about 75% of that total in the form of rain according to data from the nearest meteorological station (Aylsham) (Environment Canada, 2015b). Over the growing season, the region typically experiences a soil moisture deficit in the range of 100 to 200 mm (Carrot River Valley Watershed Association, 2013).

3.2.2 Experimental design

Soil cores were collected from existing field experiments established at the Carrot River and Arborfield sites to assess the effects of nitrification and urease inhibitors and timing of N fertilizer application on grass seed yield and N_2O emissions in the field (Yannikos, 2016). For the current study, cores were collected only from unfertilized plots and plots that had been fertilized with urea (100 kg N ha^{-1}) that was broadcast in the fall of 2011 and 2012. The field experiments were set up as a randomized complete block design, with a plot size of 11 x 12 m and 1 m spacing between all plots (Figs. 3.1 and 3.2). The plots at all 3 sites were sprayed with glyphosate at the end of the 2013 growing season in September to terminate the stand. The Carrot River plots were then split in half by cultivation with a tandem disc to impose a field tillage termination treatment.

3.2.3 Sampling protocol and storage

3.2.3.1 Arborfield site

Four intact soil cores were taken from random locations within each plot at the end of the 2013 growing season (October 8, 2013). Intact soil cores were taken by pounding a section of PVC plastic tubing (10 cm dia. x 18 cm) into the ground to remove an undisturbed column of soil of 15 cm thickness and leaving a 3 cm area at the top of the tube to which water could be added. The soil cores were returned to the University of Saskatchewan within 4 h of sampling and then frozen at -20 °C prior to starting the incubation.

3.2.3.2 Carrot river site

Sampling was done in the spring of 2014 just after spring melt once the ground was dry enough to access the sites (May 12, 2014). Three intact soil cores were collected from each plot by pounding an 18-cm length section of 10 cm dia. PVC plastic tubing into the ground to remove an intact soil core of 15 cm depth as described above. Two soil cores were collected from the non-tilled side of each plot, with one of the two cores randomly selected to undergo simulated

tillage, and the third was collected from tilled side of each plot. This provided a check for the Arborfield site where only the soil cores were 'tilled'. The soil cores were kept frozen at -20 °C until the start of the incubation.

3.2.4 Soil bulk density and particle size distribution

Following the leaching experiment, the soil cores were dried at 105 °C in a drying oven to measure the bulk density. Bulk density was calculated as the weight of oven dry soil divided by the volume of soil in the core. Once bulk density was calculated, the soil was broken up with a rolling pin and combined per block, and a representative subsample for each block was ground in a rolling mill and used to measure particle size distribution using the pipette method outlined by Kroetsch and Wang (2008).

3.2.5 Soil nitrogen

Soil inorganic N (NO_3^--N and NH_4^+-N, μg g^{-1}) was extracted using 2*M* KCl (Keeney and Nelson, 1982). For each sample, 5.00 to 5.09 g of dried, ground soil and 50 mL of 2*M* KCl solution were placed in a 250 mL high-density polyethylene (HDPE) bottle. The bottles were shaken on a rotary shaker (G10 Gyrotory Shaker, New Brunswick Scientific Co., Edison, NJ, USA) for 1h at 142 rpm and filtered through VWR 454 grade filter paper (VWR International LLC, Radnor, PA, USA) into 7-dram vials. Concentrations of soil inorganic N in the filtrate were analyzed colorimetrically using the Technicon AutoAnalyzer (Technicon Industrial Systems, Tarrytown, NY).

3.2.6 Incubation and greenhouse gas collection and measurement

3.2.6.1 Tillage simulation

At the Carrot River sites, the tillage treatments were imposed in the field with field scale tillage equipment as well as in the laboratory with a tillage simulator. For the Arborfield site, the tillage treatment was imposed only in the laboratory as the farmer at the Arborfield site did not have a disc for termination. The intact soil cores were allowed to thaw for several days at 23 °C before employing the tillage simulation. Tillage simulation was done using a custom made 4-pronged attachment for a handheld electric drill (Fig. 3.3). Each core that was selected for simulated tillage was tilled for 5 seconds at 325 rpm to a depth of 10 cm from the soil surface.

Figure 3.3 Tillage simulator attachment for handheld electric drill. The attachment consists of 4 threaded steel rods attached to 3" x 2" metal plate. This design was chosen as rod length can be changed to keep tillage depth consistent among cores.

3.2.6.2 Incubation method and sampling procedure

Each incubation was performed over a 6-week period using the method described by Hangs et al. (2013). The soil cores were kept at 23 °C during the incubation period and were kept outside the incubation chambers in trays in a 4 m x 6 m dark room between sampling days. Soil moisture content was maintained at field capacity by keeping the cores in shallow trays of water (approximately 2 cm) in between sampling days. Due to a large proportion of the Arborfield cores not sufficiently absorbing water from the tray, water was added incrementally to the top of the cores until the point that water started to drain out of the bottom. This was only needed one time and the cores remained moist for the duration of the study. The bottoms of the cores were covered with cheesecloth to minimize soil loss. In the evening before the prescribed sampling day, the cores were removed from the trays and placed on paper towel and any excess moisture was allowed to drain.

Greenhouse gas fluxes were measured by placing the cores in sealed chambers (Fig. 3.4; Nelson et al., 2007) on the sampling days and measuring the change in concentration in the chamber headspace over 1 hr. The gas-sampling chambers consisted of two 15 cm dia sections of PVC pipe glued to PVC sewer caps. All seams were sealed using silicone both inside and outside to provide an air-tight seal. The two halves of the chamber were attached using a rubber coupler and hose clamps. Two holes were drilled into the top section of each chamber: one for

installation of a rubber septum for gas sample extraction, and another to install a small fan to mix gases and prevent stratification.

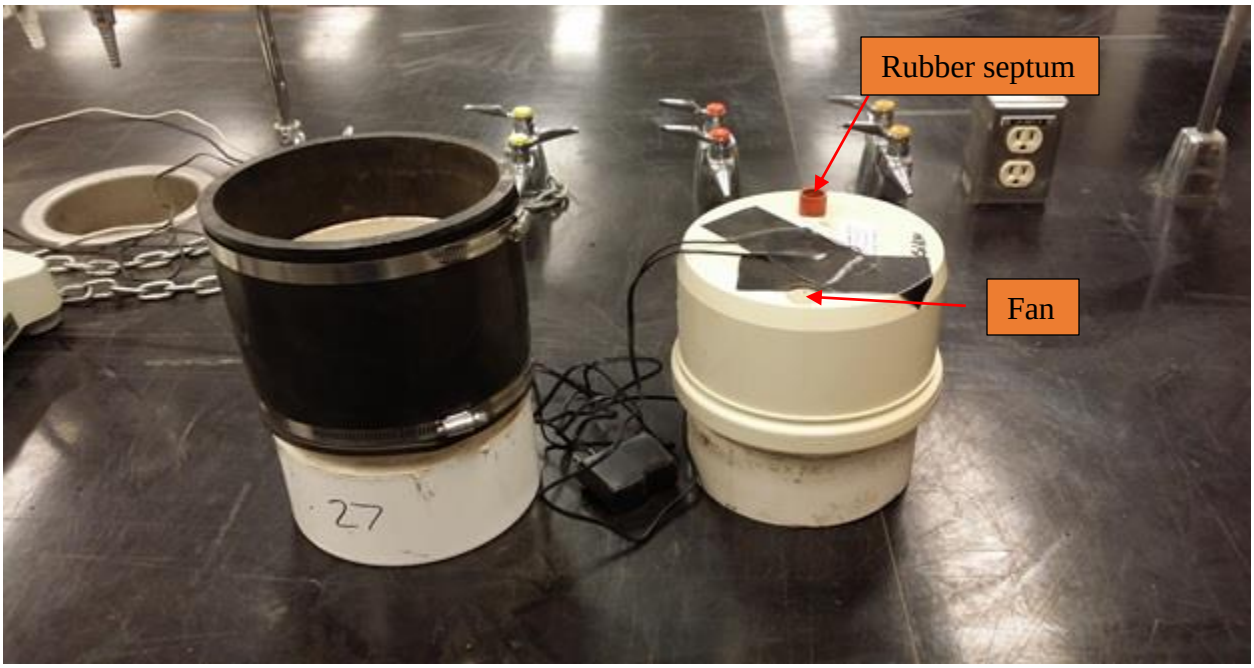

Figure 3.4 Incubation chamber used for GHG flux measurements. A small electric fan was installed in each chamber to prevent gas stratification. Samples were collected by syringe through the rubber septum on the top of each chamber.

Gas sample collection was done 1, 7, 14, 21, 28, 35, and 42 d after application of the tillage treatment. Gas samples from the chamber headspace (≈5056 cm^3) were taken at 30 and 60 min (C_{30} and C_{60}, respectively) after the chambers were sealed using a 20 mL syringe. The samples were transferred to 12 mL Exetainer™ vials (Labco Ltd., High Wycombe, UK) containing silica gel desiccant that had been evacuated to 0.00667 kPa. Four ambient air samples were collected at the start of each sampling day, with the average used as the initial concentration (C_0; Lemke et al., 1999). Carbon dioxide and N_2O concentrations were analyzed using gas chromatography on a Bruker 450-GC (Bruker Crop, Billerica, MA, USA).

Gas fluxes were estimated by calculating the concentration change in the chamber headspace for each sampling period. Flux calculations were done using the method outlined by Ginting et al. (2003). If the ratio of $(C_{30} - C_0)/(C_{60} - C_{30}) < 1$, the fluxes were calculated using linear regression. If the ratio was greater than 1, the fluxes were calculated using the Hutchinson and Mosier (1981) model modified by Agnew et al. (2010):

$$F = \frac{\rho V (C_{30}-C_0)^2}{At(2C_{30}-C_{60}-C_0)} \ln \frac{C_{30}-C_0}{C_{60}-C_{30}} \quad \text{(Eq. 3.1)}$$

Where:

F = greenhouse gas flux (mass per unit area per unit time)

ρ = density of gas (1.842 kg m^{-3} for CO_2 and 1.787 kg m^{-3} for N_2O)

V = volume of chamber headspace ($\approx$5056 cm^3)

A = cross sectional area of chamber

T = time interval (30 min)

C_0 = Average concentration of lab air

C_{30} = Concentration of sample taken at 30 min

C_{60} = Concentration of sample taken at 60 min

Assuming a constant flux between data points, cumulative emissions for each plot were estimated using linear interpolation and integrating the underlying area (Hangs et al., 2013).

3.2.7 Soil nutrient supply rate

The soil nutrient supply rate was measured during the 6-wk incubation using Plant Root Simulator™ (PRS) ion exchange resin membrane probes (Western Ag Innovations Inc., Saskatoon, SK, Canada). The probes contain either an anion or cation exchange resin held within a thin plastic frame that can be placed in soil with minimal disturbance. The advantages of using ion exchange resins over chemical extractions for nutrient ions is that they are simple, dynamic, and typically more closely correlated to plant uptake (Qian and Schoenau, 2002).

The method outlined by Hangs et al. (2004) was used for analysis and regeneration of the PRS probes. One pair of PRS probes (cation and anion) were inserted into each soil core immediately after simulated tillage was employed and were replaced weekly with recharged probes after each gas sampling period. Carefully replacing the probes in the same slot and summing the amount of nutrient supplied each week provides a reliable estimate of the cumulative supply over the whole incubation period (Qian and Schoenau, 2000a, b). At the end of each week when the probes were removed, they were scrubbed and washed with deionized

water to remove all residual soil. Once cleaned, the probes were eluted with 0.5 *M* HCl for 1h, with the eluate being analyzed for NO_3^--N, NH_4^+-N, and PO_4^{3-}-P using a Technicon Autoanalyzer II (Technicon Industrial Systems, Tarrytown, NY, USA). After the probes were eluted, they were recharged by shaking for 4h in 0.5 *M* $NaHCO_3$ three separate times.

Supply rates were calculated according to the equation:

$$SR = \frac{C \times V}{A} \quad \text{.....(Eq. 3.2)}$$

Where:

C = concentration of the eluate ($\mu g\ mL^{-1}$)

V = volume of the eluate (mL)

A = total exposed area of PRS probe membrane (16.5 cm^2)

3.2.8 Soil nutrient leaching

After the incubation was completed, the soil cores were used to assess the potential effects of the treatments on nutrient leaching. Nutrient leaching was conducted by adding 275 mL of water (equivalent to 3.5 cm of rainfall, which is a typical rainfall event for the area) to the top of each core and collecting the leachate water that passed through the core over a time period of 3 h. Leachate water was immediately filtered through a 0.4 µm polycarbonate membrane filter (EMD Millipore, Billerica, MA, USA) and frozen until analysed. The leachate was analyzed for NO_3^-, NH_4^+, and PO_4^{3-} colorimetrically as described above. Leachate data from the Arborfield cores was not used due to the leaching rates being so slow that evaporation was an issue. Multiple Arborfield cores had no appreciable leachate accumulation after 24 h and were not used in this study.

3.2.9 Statistical analysis

Statistical analysis was done using the MIXED procedure in SAS (version 9.4; SAS Institute Inc., Cary, NC, USA). The GHG emission and nutrient supply rate data were analyzed as a randomized complete block design with repeated measures, with block being a random effect and tillage and fertilization being fixed effects. Nutrient leaching, bulk density, and particle size distribution were analyzed as a randomized complete block design, with block being

a random effect and tillage and fertilization being fixed effects. PROC UNIVARIATE was used to check for normality of the data, the residuals, and the block effects. The Folded Form F statistic was used to determine if variances were equal. In cases where the data was not normally distributed, log transformation was used to achieve normality. Tukey's Honestly Significant Difference (HSD) test with an α level of 0.10 was used to compare treatment means for GHG emissions, while an α level of 0.05 was used for all other comparisons. Due to the high variability inherent in GHG emission measurements, the less rigorous alpha level was used to minimize the chance of making a type II error.

3.3 Results

3.3.1 Soil characterization

The bulk density varied among all three sites (Table 3.1), with the Arborfield cores having the highest bulk density (1.43 Mg m^{-3}) and the CR2 site at Carrot River having the lowest (1.24 Mg m^{-3}). There appears to have been some soil compaction when collecting the cores with higher clay content as their bulk densities were higher than expected based on texture alone. The bulk densities of the soil cores that were tilled in the lab all decreased compared to their non-tilled counterparts, but the decrease tended to be larger in the coarser textured Carrot River cores.

Table 3.1 Bulk density (Mg m^{-3}) of top 15 cm of soil measured on intact soil cores taken from the Arborfield (ABR) and Carrot River (CR1 & CR2) sites. Values are means (n=8).

Site	Not tilled	Lab tilled	Field tilled
	---------- Mg m^{-3} ----------		
ABR	1.43	1.42	N/A
CR1	1.35	1.29	1.35
CR2	1.24	1.19	1.25

Particle size analysis shows a marked difference in texture between the three sites (Table 3.2). The soil at Arborfield is considerably finer textured than the Carrot River soil, with lower sand content and higher silt and clay content. At the Carrot River sites, there is a slight texture gradient following the direction of water flow with silt and clay content increasing and sand content decreasing moving from CR2 to CR1.

Table 3.2 Particle size analysis of intact soil cores taken from the Arborfield and Carrot River sites in August 2013. Soil at the Arborfield site is classified as a clay loam, while soil at the Carrot River site ranges from a sandy loam to a sandy clay loam. Values are means (n=4).

Site	Sand	Silt	Clay
	---------- % ----------		
ABR	26.6	40.3	33.1
CR1	55.8	22.3	21.9
CR2	61.6	19.5	18.9

The initial soil N at the Carrot River sites (Table 3.3) showed that essentially all of the previously added urea fertilizer had been transformed or lost by the beginning of this study. The NO_3^- levels at the CR2 site tended to be higher in the 100N plots compared to the 0N plots. Conversely, the NO_3^- levels at the CR1 site were the opposite and tended to be higher in the 0N plots. These differences were not significant (p>0.05) at either site.

Table 3.3 Soil test N values at the Carrot River sites analyzed from soil collected at the beginning of the study (August, 2013). Values are means of the 4 replicates of each treatment. No significant differences were detected between any treatment at either site. Tukey's HSD was used to compare treatment means.

Site	Treatment	NO_3^-	NH_4^+
		-------- µg g^{-1} --------	
CR1	100N	5.3	4.6
CR1	0N	5.7	4.6
CR2	100N	10	4.5
CR2	0N	7.6	5.2

3.3.2 Greenhouse gas emissions

3.3.2.1 Carbon dioxide emissions

The CO_2 emissions at the Arborfield site showed moderate variation. No individual treatment produced significant differences on any given sampling day. Significant differences only appeared when comparing cumulative emissions over the entire incubation period. The cores that were not tilled with a history of fertilization produced significantly more (p<0.1) CO_2 emissions than the cores that were tilled in the lab and had no history of fertilization (Fig. 3.5).

When comparing based on fertilization history, the 100N cores produced significantly more (p<0.1) CO_2 emissions than the 0N cores, emitting 264 and 206 g CO_2-C m^{-2}, respectively (Fig. 3.6). Cultivation also had a significant effect on CO_2 emissions at the Arborfield site (Fig. 3.7), with the non-tilled cores emitting 256 g CO_2-C m^{-2} compared to 214 g CO_2-C m^{-2} in the tilled cores.

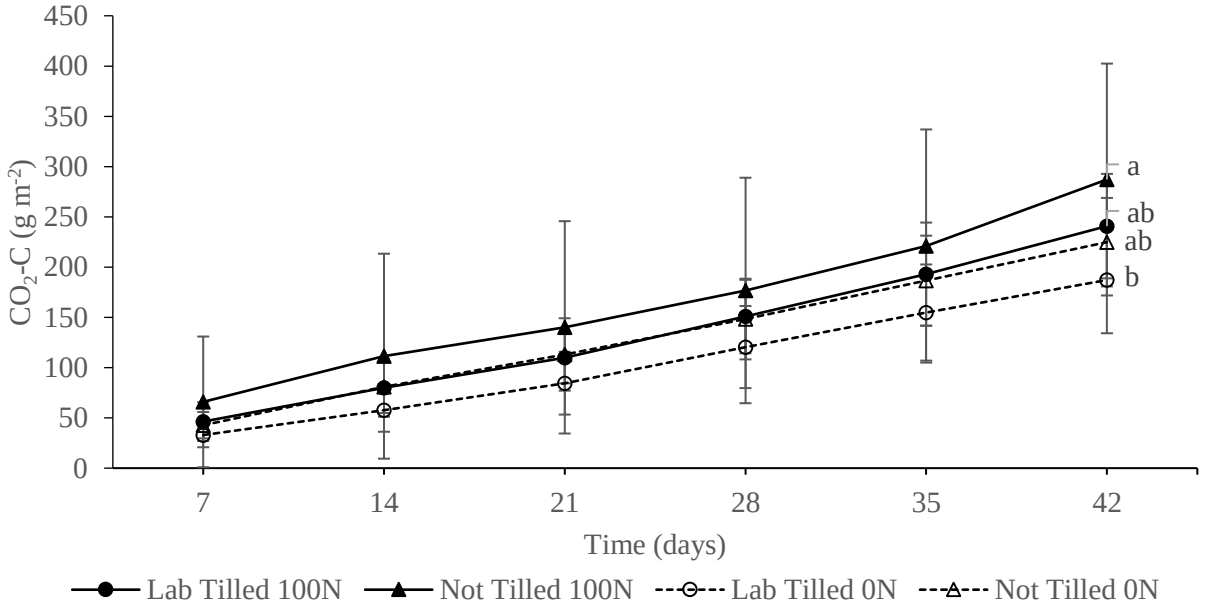

Figure 3.5 Cumulative CO_2-C emissions measured over 6 wks from intact soil cores collected following forage stand termination at the Arborfield site. Values are means from the 8 replicates of each treatment. Error bars represent one standard deviation. Letters denote significant differences (p<0.10) in cumulative emissions between treatments from repeated measures analysis. Tukey's HSD was used to compare treatment means.

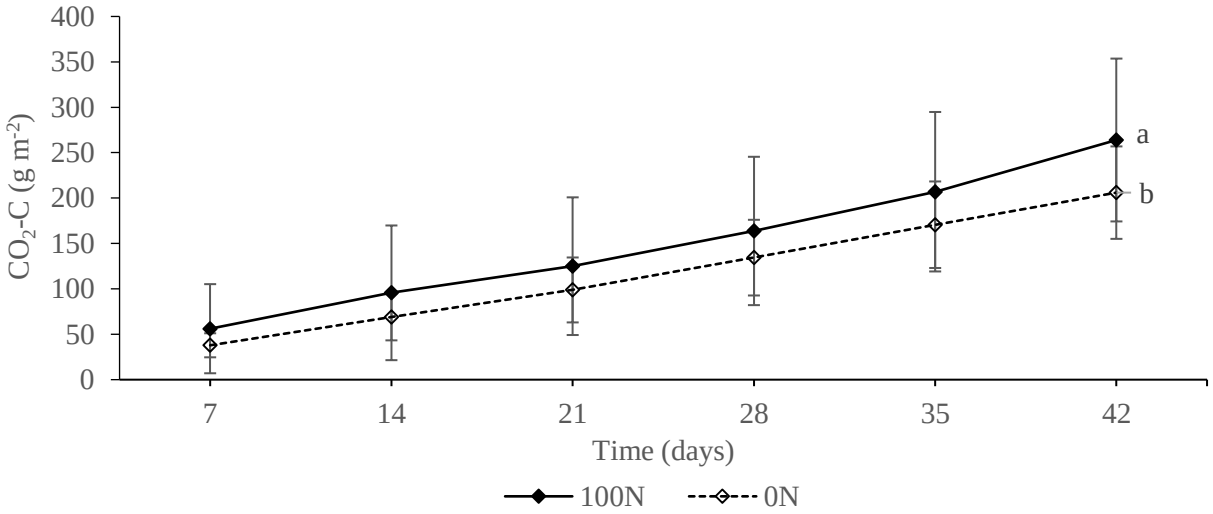

Figure 3.6 Cumulative CO_2-C emissions by fertilization treatment measured over 6 wks from intact soil cores collected following forage stand termination at the Arborfield site. Values are means from the 16 replicates of each treatment. Error bars represent one standard deviation. Letters denote significant differences (p<0.10) in cumulative emissions between treatments from repeated measures analysis. Tukey's HSD was used to compare treatment means.

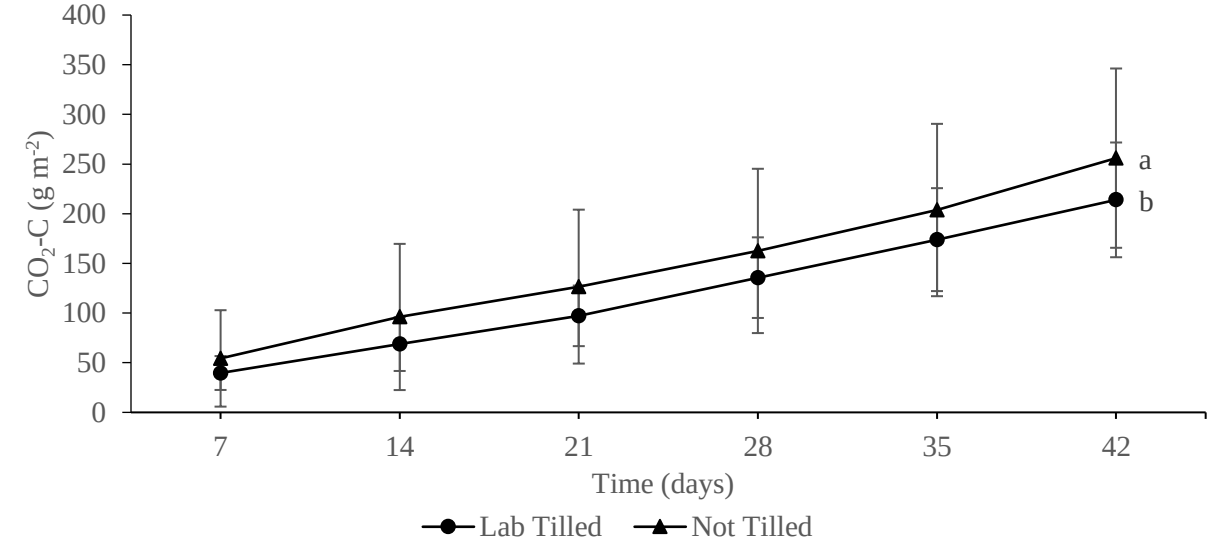

Figure 3.7 Cumulative CO_2-C emissions by tillage treatment measured over 6 wks from intact soil cores collected following forage stand termination at the Arborfield site. Values are means from the 16 replicates of each treatment. Error bars represent one standard deviation. Letters denote significant differences (p<0.10) in cumulative emissions between treatments from repeated measures analysis. Tukey's HSD was used to compare treatment means.

The CO_2 emissions at the Carrot River sites also showed moderate variation. There were no significant differences (p<0.10) between any of the treatments at the CR1 site (Fig. 3.8). Cores with a history of fertilization tended to have higher CO_2 emissions than 0N cores. Time since cultivation tended to increase emissions, with the non-tilled cores emitting the highest and the lab tilled cores emitting the lowest. Similar results are seen at the second Carrot River site (Fig. 3.9), where time since cultivation and a history of fertilization tended to slightly increase CO_2 emissions. This site had significant differences (p<0.10) between the two non-tilled core treatments, with the 0N cores emitting significantly more than the 100N cores. There were no other significant differences.

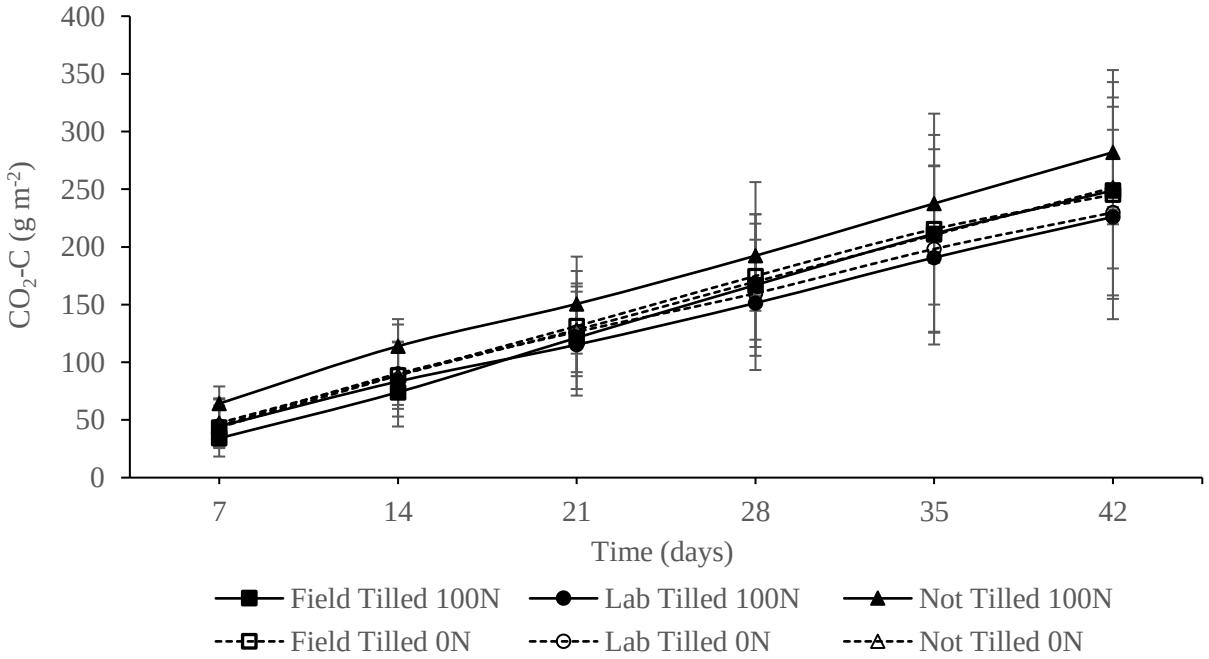

Figure 3.8 Cumulative CO_2-C emissions measured over 6 wks from intact soil cores collected in the spring following stand termination at the Carrot river site (CR1). Values are means from the 4 replicates of each treatment. Error bars represent one standard deviation. No significant differences were detected between any treatment. Tukey's HSD was used to compare treatment means.

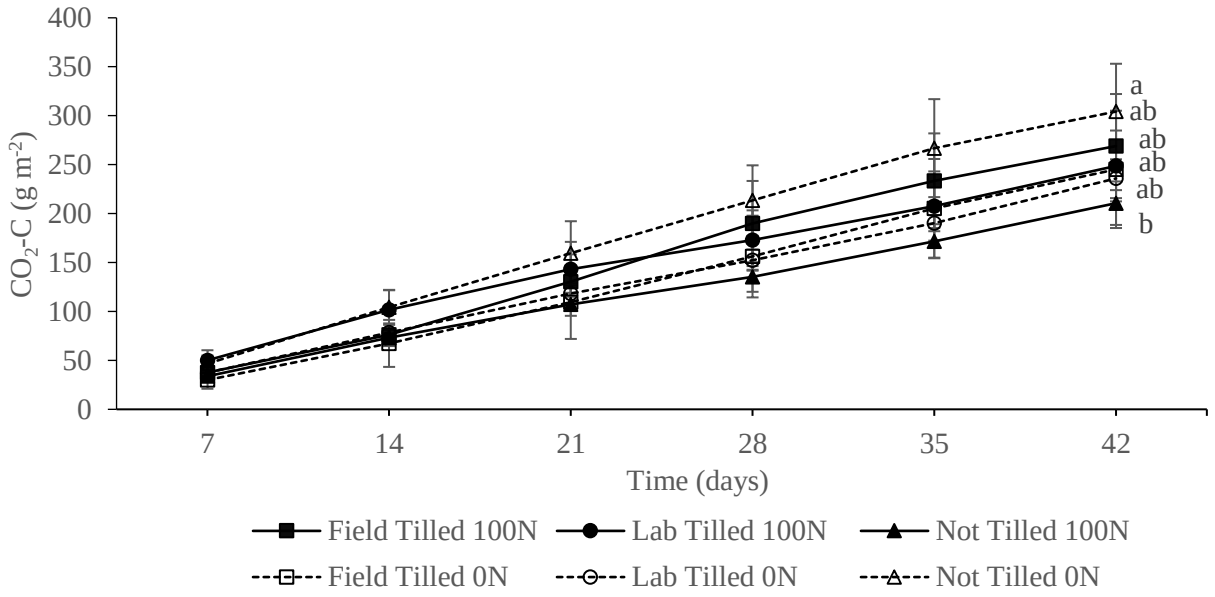

Figure 3.9 Cumulative CO_2-C emissions measured over 6 wks from intact soil cores collected in spring following Fall forage stand termination at the Carrot River site (CR2). Values are means from the 4 replicates of each treatment. Error bars represent one standard deviation. Letters denote significant differences (p<0.10) in cumulative emissions between treatments from repeated measures analysis. Tukey's HSD was used to compare treatment means.

3.3.2.2 Nitrous Oxide

Nitrous oxide emissions at the Arborfield site were extremely variable (Fig 3.10). This was mainly due to high emitting cores within each treatment. Total emissions and variability greatly increased after day 14 due to the addition of water to the tops of the cores between sampling days to maintain field capacity. No significant differences between any of the treatments were observed due to the variability, but in general fertilization history tended to increase N_2O emissions and tillage tended to decrease emissions.

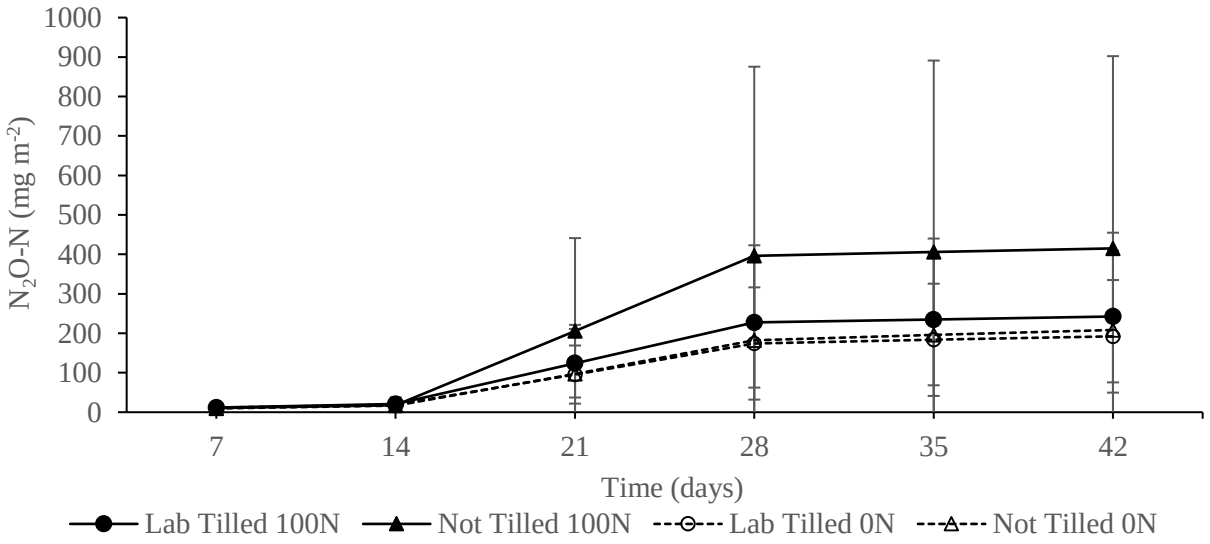

Figure 3.10 Cumulative N_2O-N emissions measured over 6 wks from intact soil cores collected following Fall forage stand termination at the Arborfield site. Values are means from the 8 replicates of each treatment. Error bars represent one standard deviation. No significant differences were detected between treatments (p<0.10). Tukey's HSD was used to compare treatment means.

Nitrous oxide emissions at the Carrot River sites were much lower and less variable than the Arborfield site. There were no significant differences between any individual treatment at either site (Figs. 3.11, 3.12). At the CR1 site (Fig. 3.13), cultivation had a significant effect (p<0.10) on N_2O emissions, with the non-tilled cores emitting nearly twice the amount of the lab tilled cores and more than twice the amount of the field tilled cores. No other significant differences were observed at the CR1 site. The cores from the CR2 site (Fig. 3.14) showed a significant difference (p<0.10) in N_2O emissions between the unfertilized cores and the cores with a fertilization history. The 100N cores emitted nearly twice the amount of N_2O as the 0N cores. No other significant differences were observed at the CR2 site.

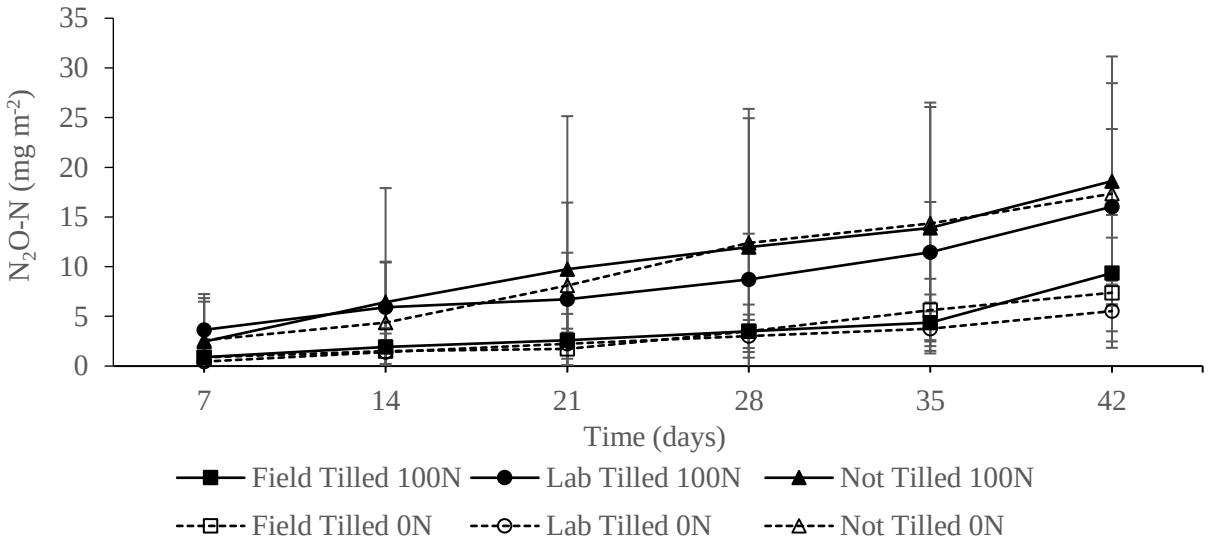

Figure 3.11 Cumulative N_2O-N emissions measured over 6 wks from intact soil cores collected in spring following Fall forage stand termination at the Carrot River site (CR1). Values are means from the 4 replicates of each treatment. Error bars represent one standard deviation. Letters denote significant differences (p<0.10) in cumulative emissions between treatments from repeated measures analysis. Tukey's HSD was used to compare treatment means.

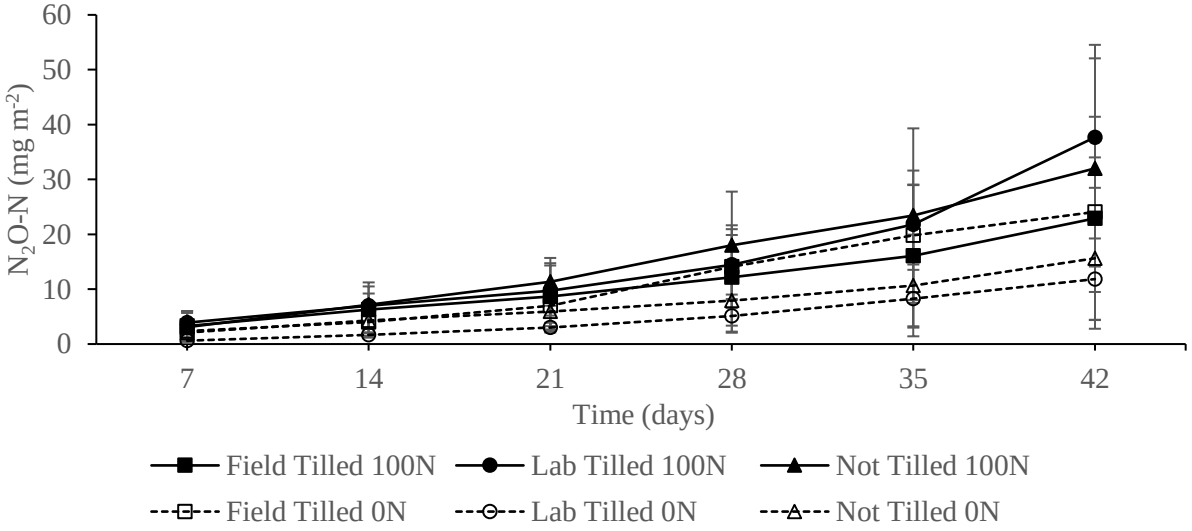

Figure 3.12 Cumulative N_2O-N emissions measured over 6 wks from intact soil cores collected in spring following forage stand termination at the Carrot River site (CR2). Values are means from the 4 replicates of each treatment. Error bars represent one standard deviation. Letters denote significant differences (p<0.10) in cumulative emissions between treatments from repeated measures analysis. Tukey's HSD was used to compare treatment means.

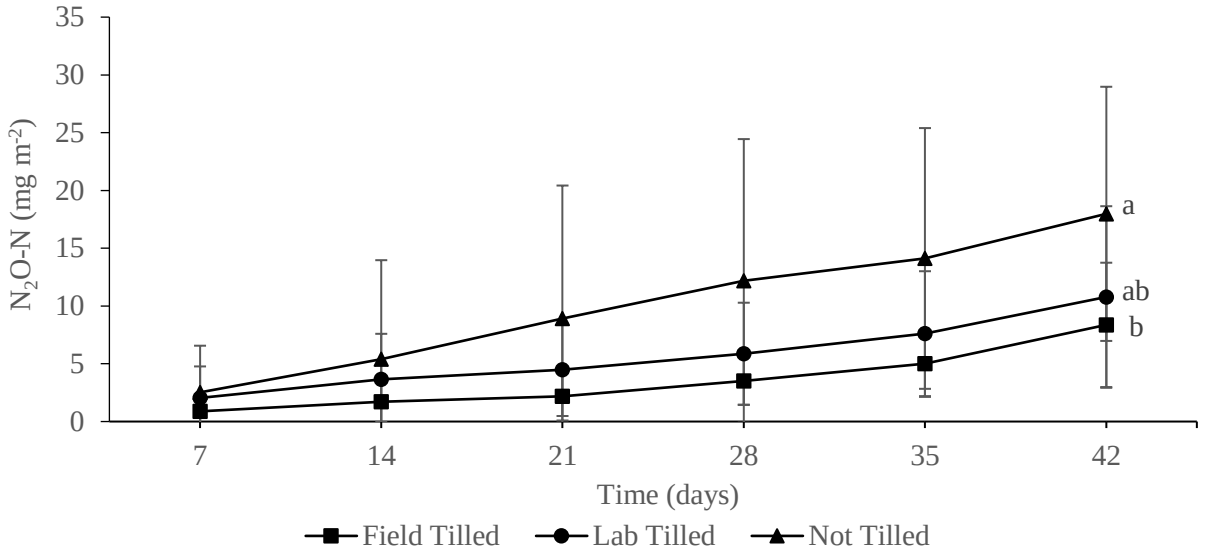

Figure 3.13 Cumulative N_2O-N emissions by tillage treatment measured over 6 wks from intact soil cores collected in spring following Fall forage stand termination at the Carrot River site (CR1). Values are means from the 8 replicates of each treatment. Error bars represent one standard deviation. Letters denote significant differences (p<0.10) in cumulative emissions between treatments from repeated measures analysis. Tukey's HSD was used to compare treatment means.

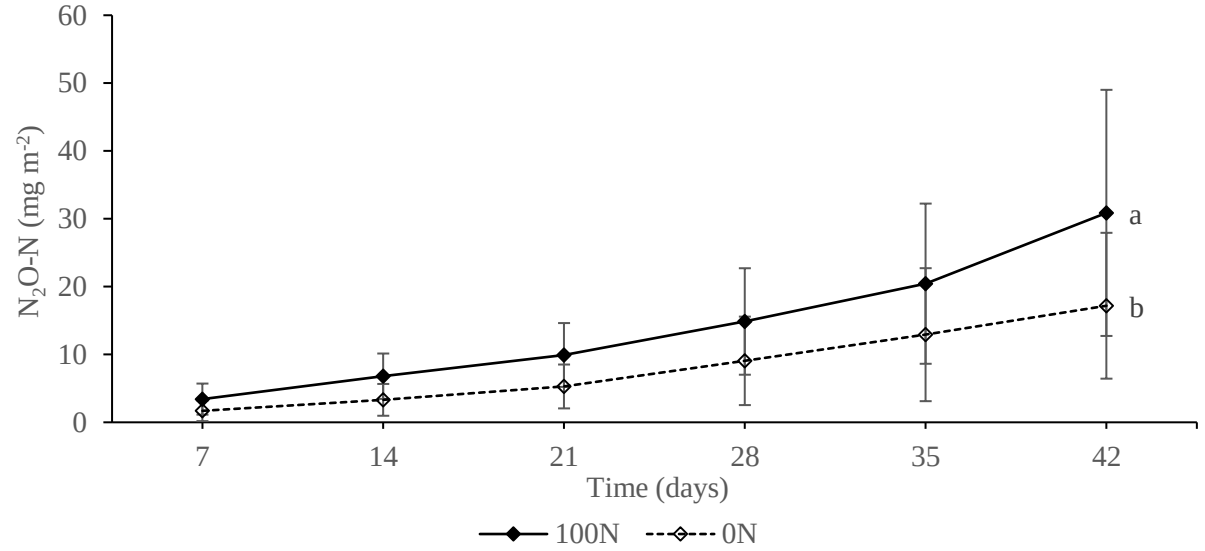

Figure 3.14 Cumulative N_2O-N emissions by fertilization treatment measured over 6 wks from intact soil cores collected in spring following forage stand termination at the Carrot River site (CR2). Values are means from the 12 replicates of each treatment. Error bars represent one standard deviation. Letters denote significant differences (p<0.10) in cumulative emissions between treatments from repeated measures analysis. Tukey's HSD was used to compare treatment means.

3.3.3 Soil nutrient supply rates

3.3.3.1 Nitrate supply rate

The NO_3^--N supply rate of the Arborfield cores (Fig. 3.15) followed a similar trend to the N_2O emissions where fertilization history tended to increase NO_3^--N supply rate and tillage tended to decrease supply rate. There was a significant difference within the 0N cores, with the non-tilled cores supplying 73% more NO_3^--N than that of the lab tilled 0N cores over the course of the incubation (22.1 vs. 12.8 µg cm^{-2}, respectively). Fertilization history did not appear to influence NO_3^--N supply rate in the non-tilled cores.

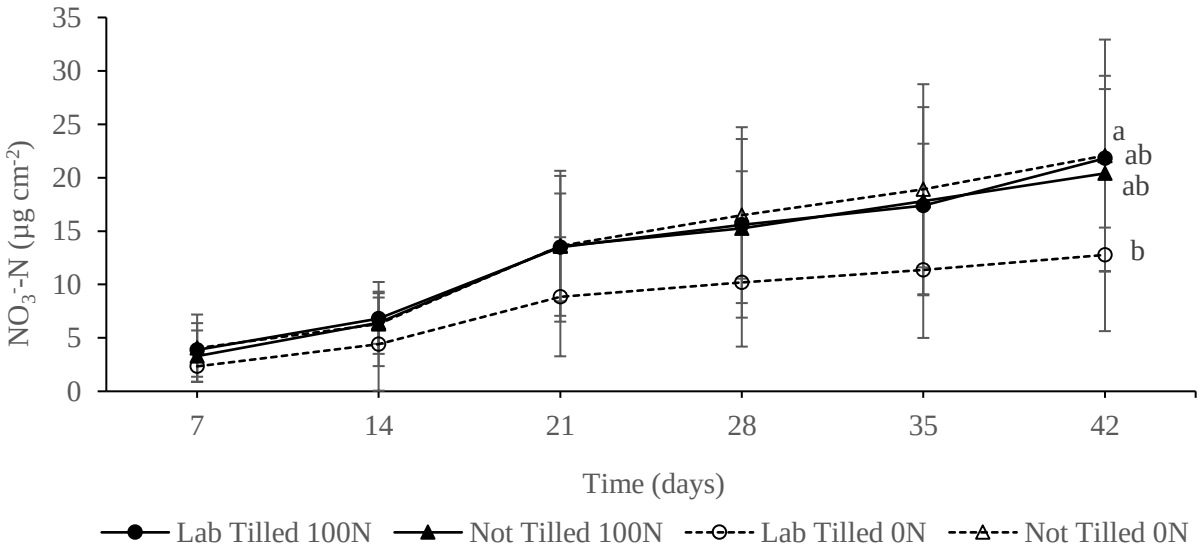

Figure 3.15 Cumulative NO_3^--N supply rate by fertilization treatment measured over 6 wks from intact soil cores collected following forage stand termination at the Arborfield site. Values are means from the 8 replicates of each treatment. Error bars represent one standard deviation. Letters denote significant differences (p<0.10) in cumulative emissions between treatments from repeated measures analysis. Tukey's HSD was used to compare treatment means.

NO_3^--N supply rate for the two Carrot River sites were similar to their respective N_2O emissions; however, there were no significant differences. At CR1 (Fig. 3.16), the non-tilled and lab tilled 100N cores tended to have the highest NO_3^--N supply rates while the 0N cores tended to have the lowest NO_3^--N supply rates. Interestingly, at CR2 (Fig. 3.17), the lab tilled 0N cores tended to have the highest NO_3^--N supply rates, and overall the 0N cores tended to have slightly higher supply rates, which was unexpected considering that the 100N plots initially had 30% more NO_3^--N than the 0N plots on average. Due to the variability within treatments, neither tillage nor fertilization history had a significant effect on NO_3^--N supply rates (p>0.05).

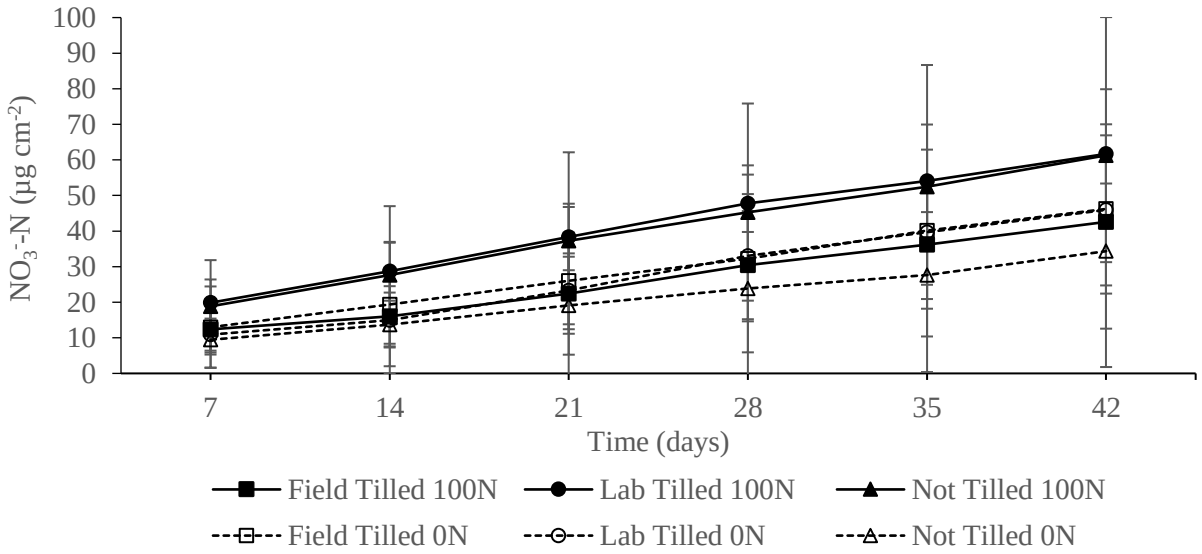

Figure 3.16 Cumulative NO_3^--N supply rate measured over 6 wks from intact soil cores collected in spring following Fall forage stand termination at the Carrot River site (CR1). Values are means from the 4 replicates of each treatment. Error bars represent one standard deviation. No significant differences were detected between treatments. Tukey's HSD was used to compare treatment means.

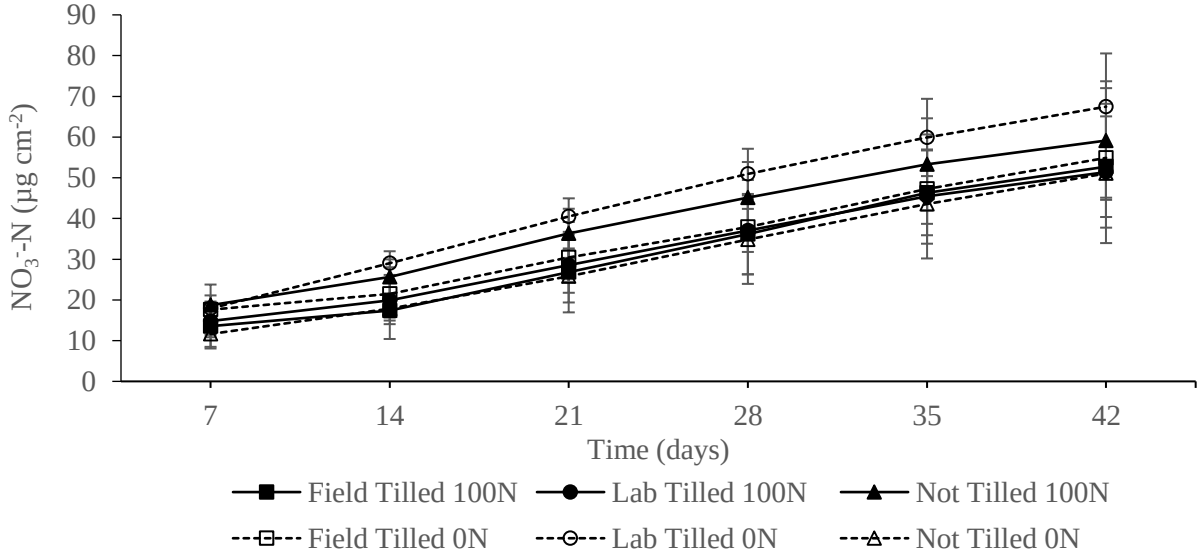

Figure 3.17 Cumulative NO_3^--N supply rate measured over 6 wks from intact soil cores collected in spring following Fall forage stand termination at the Carrot River site (CR2). Values are means from the 4 replicates of each treatment. Error bars represent one standard deviation. No significant differences were detected between treatments. Tukey's HSD was used to compare treatment means.

3.3.3.2 Ammonium supply rate

The NH_4^+-N supply rate at the Arborfield site (Fig 3.18) was nearly identical between all treatments, with no significant differences observed between any of the treatments (p>0.05). As with the N_2O emissions, the NH_4^+-N supply rates increased after the addition of water to the tops of the cores after day 14. The variability within treatments was relatively small compared to the N_2O emissions and NH_4^+ supply rates.

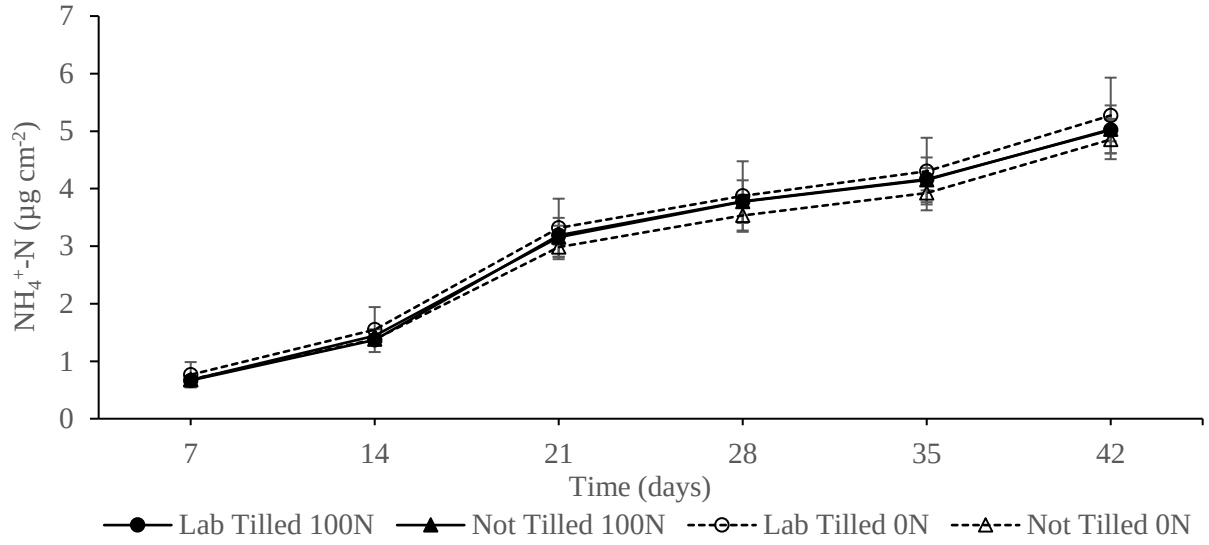

Figure 3.18 Cumulative NH_4^+-N supply rate measured over 6 wks from intact soil cores collected following forage stand termination at the Arborfield site. Values are means from the 8 replicates of each treatment. Error bars represent one standard deviation. No significant differences were detected between treatments. Tukey's HSD was used to compare treatment means.

While the differences in NH_4^+-N supply rates between individual treatments at CR1 (Fig 3.19) were not significant. However, when comparing all 100N cores to the 0N cores (Fig. 3.20), the effect on NH_4^+-N supply rates becomes significant (p<0.05), with the 100N cores supplying 15% more NH_4^+-N than the 0N cores. The supply rates measured at CR2 (Fig. 3.21) followed a similar trend to CR1 with respect to tillage, but the differences due to fertilization history were not present, and no significant differences were detected between any of the treatments.

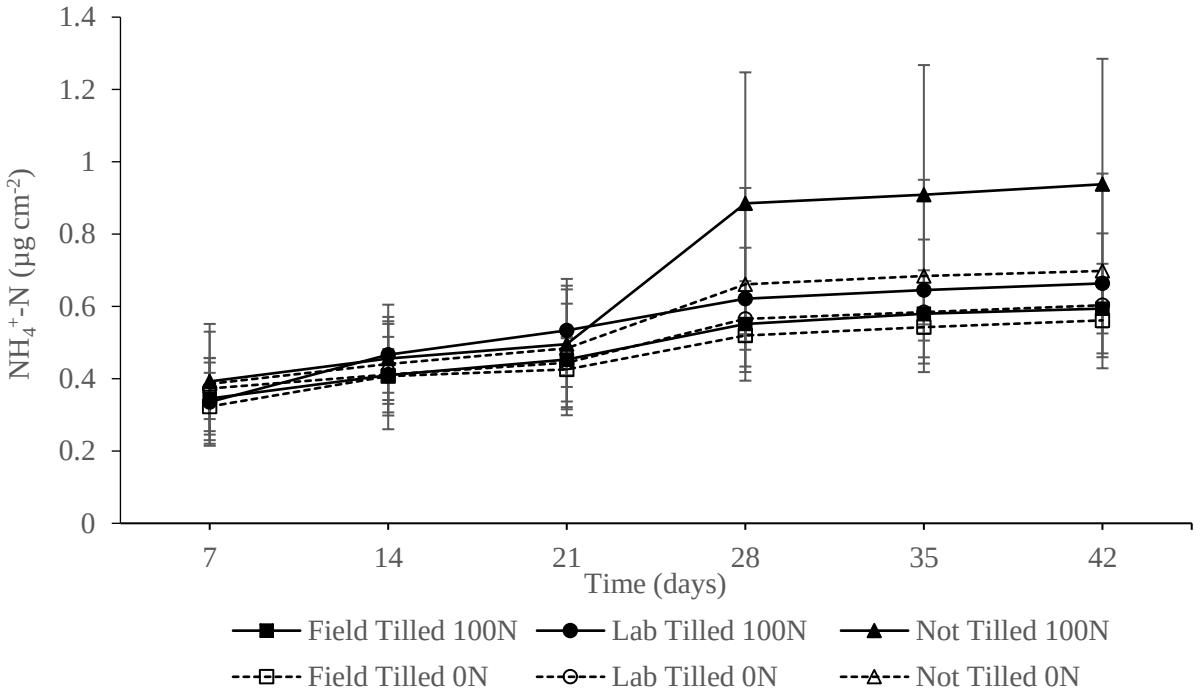

Figure 3.19 Cumulative NH_4^+-N supply rate measured over 6 wks from intact soil cores collected in spring following Fall forage stand termination at the Carrot River site (CR1). Values are means from the 4 replicates of each treatment. Error bars represent one standard deviation. No significant differences were detected between treatments. Tukey's HSD was used to compare treatment means.

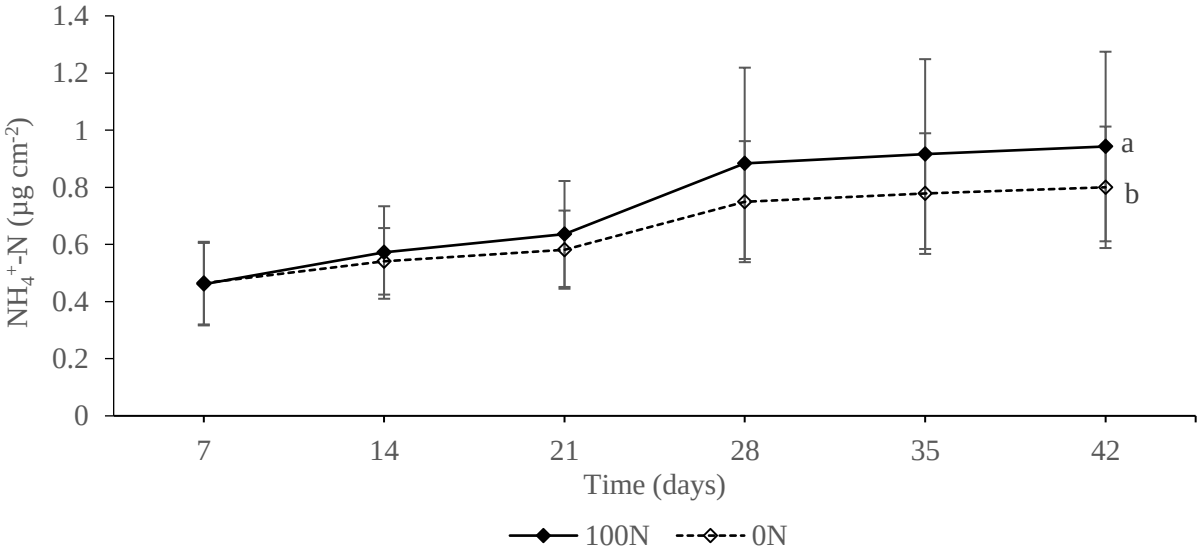

Figure 3.20 Cumulative NH_4^+-N supply rate measured over 6 wks from intact soil cores collected in spring following Fall forage stand termination at the Carrot River site (CR1). Values are means from the 4 replicates of each treatment. Error bars represent one standard deviation. Letters denote significant differences (p<0.05) in cumulative supply rate between treatments from repeated measures analysis. Tukey's HSD was used to compare treatment means.

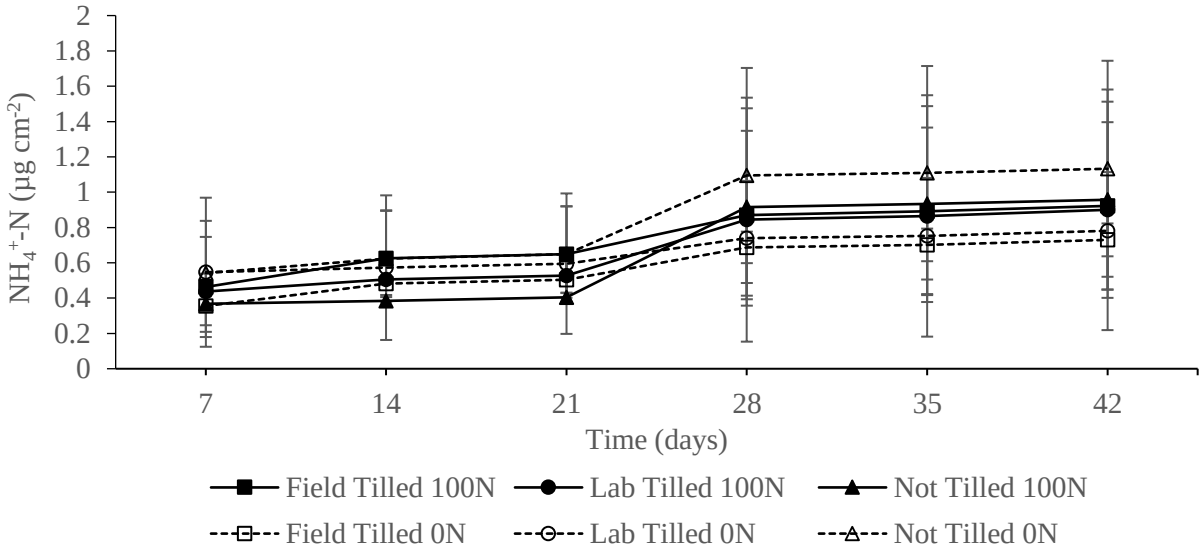

Figure 3.21 Cumulative NH_4^+-N supply rate measured over 6 wks from intact soil cores collected in spring following Fall forage stand termination at the Carrot River site (CR2). Values are means from the 4 replicates of each treatment. Error bars represent one standard deviation. No significant differences were detected between treatments. Tukey's HSD was used to compare treatment means.

3.3.3.3 Phosphate supply rate

The PO_4^{3-}-P supply rate measured in the Arborfield cores (Fig. 3.22) appeared to be slightly inhibited in the 100N cores, especially in the lab tilled 100N cores where they supplied over 30% less PO_4^{3-}-P than the non-tilled 100N cores, suggesting that the established soil structure facilitates PO_4^{3-}-P supply. While the effect is not significant, when comparing the effect of tillage on PO_4^{3-}-P supply, tillage tends to decrease supply over the entire 6 weeks.

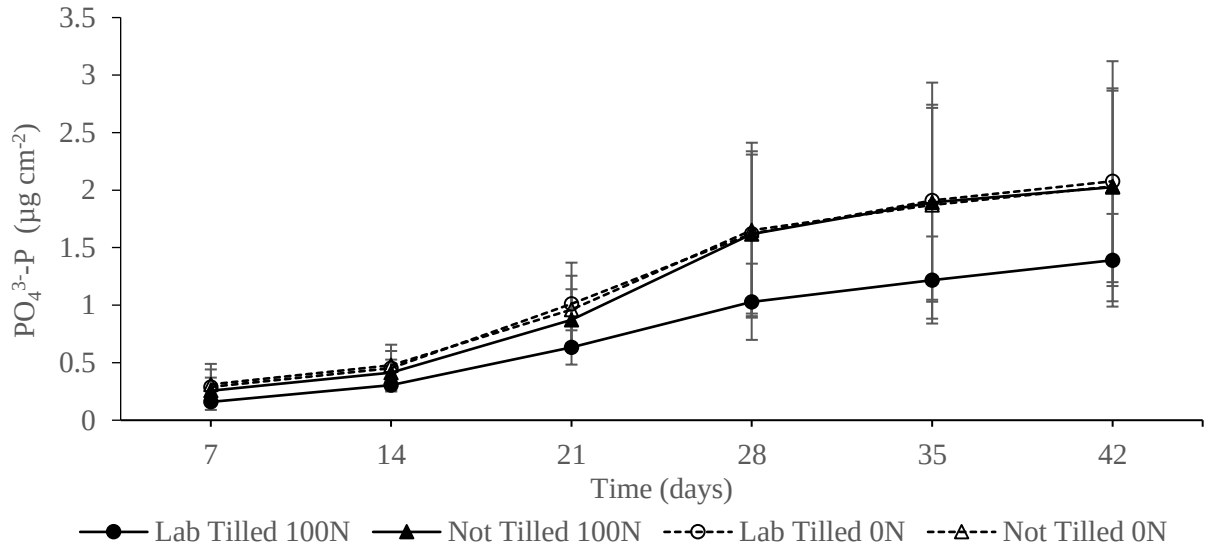

Figure 3.22 Cumulative PO_4^{3-}-P supply rate measured over 6 wks from intact soil cores collected following forage stand termination at the Arborfield site. Values are means from the 8 replicates of each treatment. Error bars represent one standard deviation. No significant differences were detected between treatments. Tukey's HSD was used to compare treatment means.

The soil cores from Carrot River further illustrated the effects of fertilizer history and tillage on PO_4^{3-}-P supply. When comparing individual treatments at CR1 (Fig. 3.23), the lab tilled 100N cores supplied the least amount of PO_4^{3-}-P at 1.21 µg cm^{-2} while the non-tilled 0N cores supplied the most at 1.74 µg cm^{-2}. The effect of fertilizer history was not as pronounced in these cores as the Arborfield cores but fertilizer history tended to decrease PO_4^{3-}-P supply rate. When examining the effect of tillage on PO_4^{3-}-P supply rates (Fig. 3.24), we can see that tillage significantly decreases the supply rate when compared to the non-tilled cores (p<0.05). The cores taken from the side of the plots that were tilled in the field land midway between the non-tilled and lab tilled cores, providing further evidence that soil structure plays an important role in PO_4^{3-}-P supply. Interestingly, the lab tilled 100N cores at the CR2 site (Fig. 3.25) had the highest PO_4^{3-}-P supply rate over the entire 6-week incubation, although the effect was not significant (p>0.05).

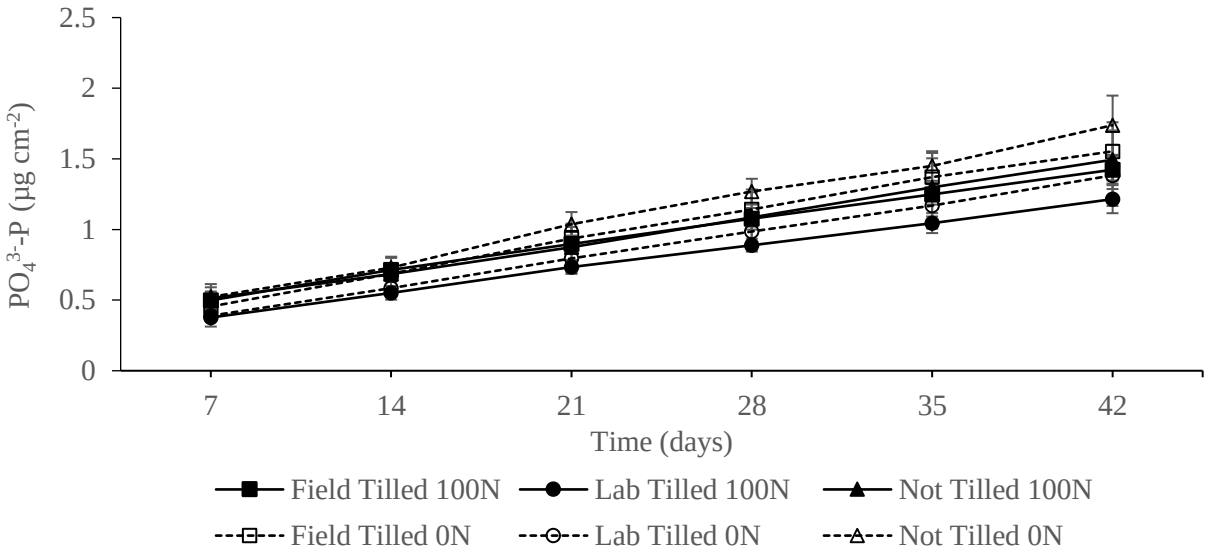

Figure 3.23 Cumulative PO_4^{3-}-P supply rate measured over 6 wks from intact soil cores collected in spring following Fall forage stand termination at the Carrot River site (CR1). Values are means from the 4 replicates of each treatment. Error bars represent one standard deviation. No significant differences were detected between treatments. Tukey's HSD was used to compare treatment means.

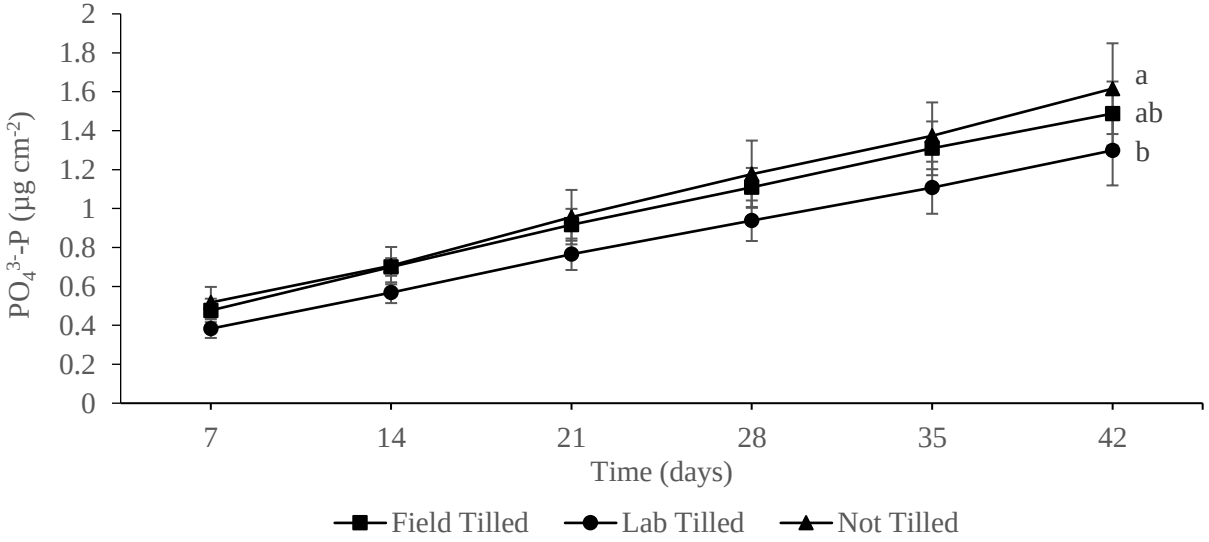

Figure 3.24 Cumulative PO_4^{3-}-P supply rate measured over 6 wks from intact soil cores collected in spring following Fall forage stand termination at the Carrot River site (CR1). Values are means from the 8 replicates of each treatment. Error bars represent one standard deviation. Letters denote significant differences (p<0.05) in cumulative supply rate between treatments from repeated measures analysis. Tukey's HSD was used to compare treatment means.

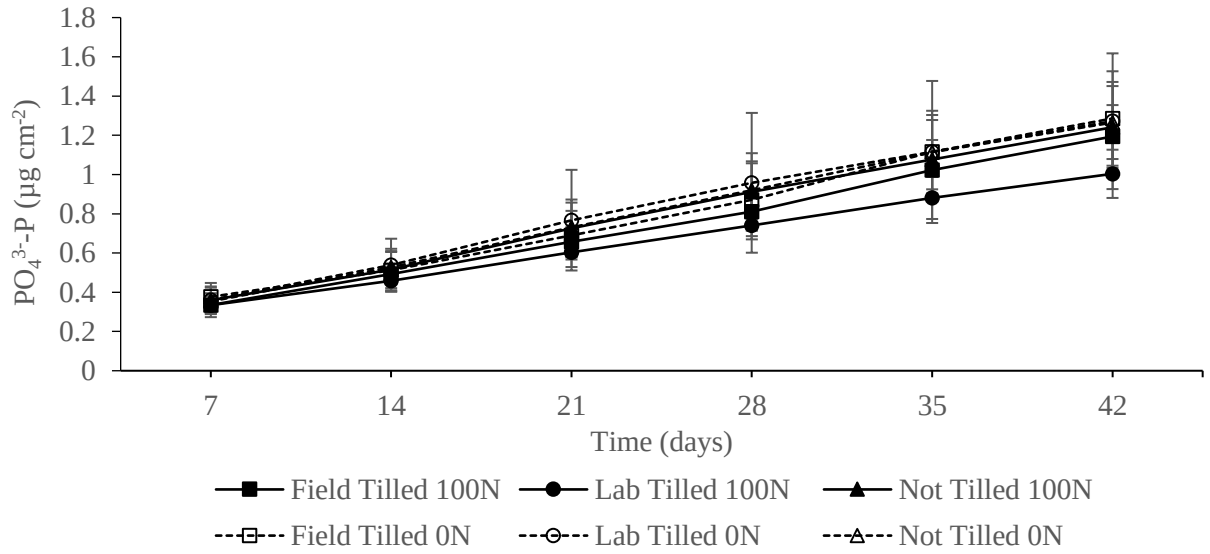

Figure 3.25 Cumulative PO_4^{3-}-P supply rate measured over 6 wks from intact soil cores collected in spring following Fall forage stand termination at the Carrot River site (CR2). Values are means from the 4 replicates of each treatment. Error bars represent one standard deviation. No significant differences were detected between treatments. Tukey's HSD was used to compare treatment means.

3.3.4 Soil nutrient leaching

When examining leaching rates within cores from the Carrot River sites, fertilization history significantly increased the amount of NO_3^- leached compared to the unfertilized plots at CR1 (Table 3.4), with 2330 vs. 1160 µg kg^{-1} leached respectively. In general, fertilization history tended to increase the amount of NH_4^+ and NO_3^- and slightly decrease the amount of PO_4^{3-} that leached out of the cores (Tables 3.5 and 3.6). There were no other significant differences (p>0.05) recorded between any treatment at either site.

Table 3.4 Comparison of N fertilizer history on the amount of NH_4^+, NO_3^-, and PO_4^{3-} leached per kg of soil from intact soil cores (CR1) after the addition of 3.5cm of water. Values are means from the 12 replicates of each treatment. Letters within columns denote significant differences (p<0.05). Tukey's HSD was used to compare treatment means.

Treatment	NH_4^+	NO_3^-	PO_4^{3-}
	---------- µg kg^{-1} ----------		
100N	19.9a	2330a	32.7a
0N	12.1a	1160b	32.5a

Table 3.5 Amount of NH_4^+, NO_3^-, and PO_4^{3-} leached per kg of soil across all treatments from intact soil cores (CR1) after the addition of 3.5cm of water. Values are means from the 4 replicates of each treatment. No significant differences were detected between treatments. Tukey's HSD was used to compare treatment means.

Treatment	NH_4^+	NO_3^-	PO_4^{3-}
	---------- µg kg^{-1} ----------		
Field Tilled 100N	19.3	1820	31.5
Lab Tilled 100N	17.9	2380	30.1
Not Tilled 100N	22.6	2790	36.3
Field Tilled 0N	10.9	791	34.5
Lab Tilled 0N	10.2	1150	24.9
Not Tilled 0N	15.3	1530	38.0

Table 3.6 Amount of NH_4^+, NO_3^-, and PO_4^{3-} leached per kg of soil across all treatments from intact soil cores (CR2) after the addition of 3.5cm of water. Values are means from the 4 replicates of each treatment. No significant differences were detected between treatments. Tukey's HSD was used to compare treatment means.

Treatment	NH_4^+	NO_3^-	PO_4^{3-}
	---------- µg kg^{-1} ----------		
Field Tilled 100N	9.8	359	41.0
Lab Tilled 100N	16.4	1350	32.3
Not Tilled 100N	15.4	892	60.6
Field Tilled 0N	15.9	344	39.3
Lab Tilled 0N	16.9	924	49.4
Not Tilled 0N	13.2	652	43.0

3.4 Discussion

3.4.1 Greenhouse gas emissions

Overall, CO_2 emissions between the two sites were nearly identical, with the Arborfield cores producing an average of 235 g m^{-2} CO_2-C while the Carrot River cores produced 247 and 250 g m^{-2} CO_2-C at CR1 and CR2, respectively. Nitrous oxide emissions on the other hand differed greatly between the two sites, with the Arborfield cores emitting nearly 15 times more N_2O than the Carrot River cores with an average of 265 mg m^{-2} N_2O-N and the CR1 and CR2

cores emitting 12.4 and 24.0 mg m^{-2} N_2O-N, respectively. By day 14 the Arborfield cores already emitted nearly the same amount of N_2O-N as the Carrot River cores did over the entire 6 weeks. This stark difference in magnitude is mostly due to the water that was added to the Arborfield cores after day 14. Another possible explanation for the higher N_2O-N emissions in the Arborfield cores is related to clay content and bulk density. Skiba and Ball (2002) reported that N_2O emissions were positively influenced by increasing clay content and bulk density, and the Arborfield cores contained over 60% more clay and had over 10% higher bulk density compared to the Carrot River cores (Tables 3.1 & 3.2).

Soil cores that were not tilled across all three sites consistently had the highest CO_2-C emissions over the 6-week incubation, with the exception of the non-tilled 100N cores at CR2 (Fig. 3.8). This was significant at the Arborfield site, where the non-tilled cores emitted 256 g m^{-2} and the tilled cores emitted 214 g m^{-2} over the entire 6 weeks (Fig. 3.7). The results were similar in the measured N_2O-N fluxes, where the non-tilled cores tended to have increased production, especially at the CR1 site where the non-tilled cores produced significantly more N_2O-N than the tilled cores (17.98 vs. 10.78 mg m^{-2}) (Fig. 3.13). It appears that destruction of the structure in these soils may be inhibiting GHG production. A study in Nottinghamshire, UK by Mangalassery et al. (2013), found that CO_2 and N_2O emissions were significantly influenced by soil texture and aggregate size. In a clay loam, they found that a well-developed structure with large aggregates produced the highest CO_2 and N_2O flux. A similar trend was seen in the sandy loam textured soil, where larger aggregates produced greater CO_2 and N_2O fluxes. The aggregate class with the next highest emissions was typically the finest, measuring <0.5 mm, suggesting that very intensive tillage could significantly increase CO_2 and N_2O emissions in these soils.

Rochette et al. (2008) reported in eastern Canada that no-till more than doubled N_2O emissions in a heavy clay soil over moldboard plowing. While the effect was greatest immediately after tillage, they also observed lower N_2O emissions the following spring and summer, indicating that tillage in fine textured soils rich in organic matter provides a depressing effect for a significant amount of time. This drawn out depressive effect can be seen at CR1, where the field tilled cores produced significantly less N_2O than the non-tilled cores (Fig. 3.13).

The effect of fertilization history on the greenhouse gas emissions was more pronounced than the tillage effect, where fertilization had an overall positive effect on emissions. This is exemplified in the CO_2-C emissions from the Arborfield cores (Fig. 3.6), where the 100N cores

produced significantly more (p<0.1) at 264 g m^{-2} compared to the 206 g m^{-2} the 0N cores produced over the 6 weeks, and the N_2O-N emissions from the CR2 cores (Fig. 3.14), with the 100N cores producing 30.9 mg m^{-2} while the 0N cores produced significantly less at 17.2 mg m^{-2}. A larger amount of N_2O emissions in previously fertilized plots is to be expected, especially in fields such as the ones in this study where saturation and flooding are regular occurrences in the spring after snow melt.

In a study by Venterea et al. (2005), they concluded that the effect of tillage on N_2O emissions is influenced by the type of fertilizer used. In systems that used anhydrous ammonia injection, N_2O emissions were higher under conventional tillage, but when broadcast urea was used, as was done in the lead up to this study and is common practice in the forage industry, no till systems produced significantly more N_2O.

Differences in N_2O emissions were difficult to measure due to the high variability within treatments, but also because the initial soil N levels were very similar between the 100N and 0N plots. At the time when the initial soil samples were collected, it had already been approximately 12 months since the last fertilizer application. By this point, there were no significant differences in NO_3^- or NH_4^+ at either Carrot River site (Table 3.3).

3.4.2 Nutrient supply rates

Like the N_2O emissions, most nutrient supply rates were highly variable, causing there to be few significant differences. There were no significant differences in any of the measured NO_3^--N supply rates, but in general the 100N cores tended to have slightly higher supply rates. In the Arborfield cores (Fig. 3.15), values ranged from 12.8 to 22.1 µg cm^{-2} over the 6-week incubation, with the lab tilled 0N cores being the lowest. Within the Carrot River cores, CR1 (Fig. 3.16) ranged from 34.3 to 61.3 µg cm^{-2}, while CR2 (Fig. 3.17) ranged from 51.1 to 67.5 µg cm^{-2} and in both cases the non-tilled 0N cores were the lowest.

The NH_4^+-N supply rates were considerably less variable than the NO_3^--N supply rates. The Arborfield cores had very similar NH_4^+-N supply rates between all treatments, ranging from 4.85 to 5.27 µg cm^{-2} (Fig. 3.18). The supply rate of NH_4^+-N appeared to be more responsive to the wetting that occurred in the Arborfield cores after day 14 compared to the NO_3^--N supply rate from the same cores. The Carrot River cores had much lower overall NH_4^+-N supply rates compared to the Arborfield cores, only reaching a maximum of 1.21 µg cm^{-2} (Figs. 3.19 & 3.21).

There is a significant difference ($p<0.05$) in NH_4^+-N supply rate when comparing the 100N cores to the 0N cores at the CR1 site, with the 0N cores supplying 0.80 µg cm^{-2} and the 100N cores supplying 0.94 µg cm^{-2} (Fig. 3.20).

Overall, the Carrot River 100N cores tended to have higher N supply rates than the 0N cores over the course of the incubation, even though the initial soil tests showed no significant difference between the amount of NH_4^+ or NO_3^- (Table 3.3). This suggests that the 100N plots have accumulated a pool of potentially mineralizable organic N, leading to the higher N supply rates over the 0N plots. A similar result was obtained in a study by Hangs et al. (2013), where a high N supply rate in a N-limited plot was attributed to a build up of potentially mineralizable fraction of soil organic N.

The PO_4^{3-}-P supply rates generally had a negative response to tillage, but fertilization history also had a small inhibitory effect. Within the Arborfield cores (Fig. 3.19), values ranged from 1.39 µg cm^{-2} in the lab tilled 100N cores, to 2.07 µg cm^{-2} in the lab tilled 0N cores. The non-tilled cores had virtually the exact same PO_4^{3-}-P supply rate over the 6-week incubation at 2.03 µg cm^{-2}. The PO_4^{3-}-P supply rates from the Carrot River cores (Figs. 3.23 & 3.25 respectively) followed a similar trend to the cores from Arborfield with the exception of the lab tilled 100N cores at CR2, which had the highest supply rate in the group. Within the CR2 cores, tillage has a significant inhibitory effect on PO_4^{3-}-P supply rate (Fig. 3.24) with the lab tilled cores having a significantly lower ($p<0.05$) rate compared to the non-tilled cores. The field tilled cores had some time in between when tillage was performed and the soil cores were collected, suggesting that soil structure plays an important role in phosphorus availability. The effect of tillage on PO_4^{3-}-P supply was seen in a study done by Messiga et al. (2009) where they compared soil phosphorus availability in no-till versus conventional tillage following freezing and thawing cycles. After a single freeze thaw cycle, the no-till soils averaged 17.4 mg kg^{-1} P while the conventional till soils averaged only 7.5 mg kg^{-1}.

3.4.3 Nutrient leaching rates

The nutrient leaching rates rarely showed significant differences between any treatment with either set of Carrot River cores. This was partly due to there being no significant differences in initial nutrient concentrations at both sites but was also influenced by a high variability in concentrations in the leachate water. This was especially apparent with NO_3^--N, where the

highest value was as much as 725 times greater than the lowest value within a single treatment. These extreme ranges are possibly due to preferential channels forming on the sides of the cores from the soil residue, allowing water to pass by the soil rather than through it.

When comparing fertilized to unfertilized cores at CR1 (Table 3.4), there was a significantly higher concentration of NO_3^--N that leached out of the 100N cores compared to the 0N cores. In general, no-till tended to increase the amount of all nutrients leached, while fertilization history tended to increase N leaching while very slightly decreasing phosphorus leaching.

3.5 Conclusions

This chapter studied the response of greenhouse gas emissions, nutrient supply rates, and nutrient leaching to past and present management practices, and it suggests that in high clay, wet soils like the ones examined here, tillage may reduce soil greenhouse gas emissions and nutrient loss through leaching after the removal of an established forage stand. The trade-off is that tillage generally reduces nutrient supply rates. On the other hand, N fertilizer in the form of broadcast urea typically increases all greenhouse gas emissions, N leaching rates, but slightly decreases phosphorus leaching and supply rates.

Across both the Arborfield and Carrot River sites, termination by tillage was associated with an overall reduction in CO_2 as well as N_2O emissions. The largest reduction in greenhouse gas emissions in response to tillage occurred in CO_2 emissions at the Arborfield site and N_2O emissions from cores taken from the seasonally waterlogged side of the Carrot River field (CR1). While the other greenhouse gas reductions were not statistically significant, with more reps one would likely tease out more significant differences. The effect of tillage on nutrient supply rates were generally negative but were usually small and not statistically significant. Tillage also tended to decrease nutrient leaching rates, but again the effect was generally small and not significant.

Unsurprisingly, prior applications of fertilizer significantly increased CO_2 and N_2O emissions. It also tended to increase N supply rates, especially NH_4^+ at CR1 where the supply rate was increased by approximately 18% over the unfertilized cores. The downside is that N fertilization tended to decrease PO_4^{3-} supply rate, although the effect was not statistically

significant. Nitrogen leaching rates were significantly higher in the 100N plots at CR1 with a similar trend seen at CR2.

In the future, a similar study would benefit from taking more regular gas samples in the field and for a longer period of time to see how quickly significant soil structure returns after tillage and if the differences disappear. The moment right after termination likely has a significant greenhouse gas flux, as well as immediately after spring thaw when the field floods. Furthermore, increased repetitions would also improve the likelihood that significant differences would be spotted between both fertilization and termination method.

One thing this study did not take into account was the greenhouse gas emissions from the equipment used for physically tilling the soil. It is highly likely that the small differences in soil greenhouse gas emissions would disappear due to emissions from the equipment.

4.0 The Influence of Tillage and Fertilization History on Soil Carbon Fractions in Two Grass Fields in Northeastern Saskatchewan

4.1 Introduction

At an estimated 2500 gigatons (Gt), soils contain the largest terrestrial global pool of C, more than 3 times larger than the atmospheric pool and 4.5 times larger than the biotic pool (Lal, 2004). The soil C is concentrated near the surface, with approximately 90% of the soil C pool residing within the top 100 cm of soil, and on average 39-70% within the top 30 cm (Batjes, 2014). Due to soil C being a highly active pool exchanging C with the atmosphere it plays a significant role in the global C cycle and is an important consideration in climate change.

Soil C is comprised of organic and inorganic forms. The inorganic forms are largely carbonates, while SOC represents the active portion of the soil C pool and is estimated to contain over 1500 Gt of C (Lal, 2004). The massive extent of this pool and its highly active nature make it particularly sensitive to homeostatic changes. The various pools of SOC are constantly changing, depending on vegetation type and amount, climate, precipitation, and land use change. Pools that change over hundreds or thousands of years are often termed "stable" pools, while those that may be altered significantly in shorter time periods (decades, a few years, months or days) are termed "labile". Conversion of native land to agriculturally productive land is estimated to have created a global soil C debt of approximately 133 Gt, with the rate of loss significantly increasing over the last 200 years (Sanderman et al., 2017). It is therefore paramount that good land management techniques are employed to slow or reverse soil C loss.

Soil labile C, which is C that is readily oxidizable, turns over quickly and is more responsive to management changes than the total SOC. The labile fraction of SOC is mainly comprised of particulate organic C, dissolved organic C, microbial biomass C, and other easily oxidizable C and these fractions are typically used as early sensitive indicators of management practices on soil quality (Li et al., 2011) as it relates to soil organic matter.

Light fraction organic carbon represents a portion of particulate organic C that is non-humified and is mainly composed of recently deposited plant residues as well as older inactive material originating from plant debris that is less that 2mm in size. Light fraction organic carbon is an important pool to consider when examining the effects of land management changes on SOC. Larney et al. (1997) reported that LFOC concentrations were 30 – 55 % lower in systems that included fallow compared to a continuous wheat system in a long-term study in southern Alberta. Approximately one-third of the total SOC decrease was attributed to the reduced LFOC from the fallow systems, leading them to conclude that LFOC is the most robust indicator of management induced effects on SOC. While LFOC has a wide C:N ratio, it typically decomposes faster than whole organic matter, acting as a source of nutrients and substrate for microbial processes. The LFOC portion of TOC can be increased through reduced soil disturbance, increased crop residue, fertilization, and use of perennial forages (Malhi et al., 2003). Increased moisture and temperature, as well as other factors that increase decomposition, decrease the amount of LFOC in soils and the balance between these forces influence the amount of LFOC at equilibrium in a given soil.

The most active and mobile fraction of SOC is the C that is dissolved in the soil solution. The organic C dissolved in soil solution is readily decomposable and able to move large distances with water through the soil profile. Despite dissolved organic C (DOC) being a relatively small pool of organic C, it is now recognized to significantly influence soil biological activity, metal and organic pollutant transport, mineral weathering, and podzolization (Chantigny, 2003). Water extractable organic carbon is typically used as a surrogate for DOC, as measuring DOC *in situ* through extraction of pore water is laborious, and extraction with concentrated salt solutions causes additional C release through desorption and dissolution (Chantigny et al., 2008). The WEOC is typically influenced by land management practices that alter the physical and chemical properties of the soil, such as tillage and N fertilization. However, the main influence on WEOC concentrations comes from root turnover and rhizodeposition from grass species (Nguyen, 2003; Schwendenmann and Veldkamp, 2005).

The living component of soils, consisting mostly of bacteria and fungi (microbial biomass), are responsible for the decomposition of biotic residues and contribute to nutrient cycling within the soil profile. Due to its high turnover rate and acting as an immediate sink for soil nutrients, it plays an active role in nutrient transformation (Parham, 2013). Microbial

biomass primarily accrues close to the soil surface, and is typically greater in no-till systems, although this is not always the case (Franzluebbers et al.,1994; Drijber at al., 2000; Helgason et al., 2009). Fertilizer N application tends to increase microbial biomass in the soil, likely due to greater plant production, which increases root exudates and crop residues, and increases nutrient availability (Lupwayi et al., 2010).

The objectives of the study described in this chapter is 1) to determine the effect of grass forage stand termination method on the various soil C fractions and 2) assess the influence of contrasting N fertilization histories on the soil C fractions at the Carrot River SK location. It is hypothesized that N fertilization history along with tillage as termination method will increase the labile soil C fractions measured after the grass stand has been terminated for use in annual crop production. The sites and treatments used for this study of soil C forms and distribution have been described in detail in the previous chapter on greenhouse gas production effects. Therefore, only a brief description follows.

4.2 Materials and Methods

4.2.1 Site characteristics

The soil samples for C analysis were collected from the two sites at the Carrot River location (Fig. 3.2) described in Chapter 3 (CR1, CR2). The field in which the two sites are located is on the edge of the boreal forest in the Dark Gray soil zone. Briefly, the CR1 site is located along the northern side of the field, while CR2 is located in the southwestern corner. Gronlid association Gleyed Rego Dark Gray and Gleyed Calcareous Dark Gray Chernozems dominate the landscape, with upper slopes having a mix of Carrot River association Gleyed Dark Gray and Gleyed Calcareous Dark Gray Chernozems, and lower slopes having mainly Gronlid association Gleyed Dark Gray Chernozems (CanSIS soil survey, 1997a). The texture of the field is a sandy clay loam to sandy loam and the topography is mostly level with a slight downward slope to the northeast corner of the field. Hybrid bromegrass [*Bromus inermis* Leyss. (L.) *x Bromus riparius* Rehm. (L.)], variety ‘Success’, was planted in 2010 and the stand was terminated at the end of 2013. At the end of each growing season, the seed was harvested, the residue was baled, and the remaining stubble was burned at the start of the following season as is the normal practice for bromegrass production in the area.

4.2.2 Experimental design

As described in Chapter 3, the study used for this thesis work is a continuation of an experiment designed to assess the effects of nitrification and urease inhibitors as well as fertilizer timing on seed yield and N_2O emissions from the bromegrass stands. Only plots fertilized with fall broadcast urea and unfertilized control plots were used for the current study. The plots are approximately 11 x 12 m with 1 m spacing between plots on all sides and were organized in a randomized complete block design. At the end of the 2013 growing season, all plots were sprayed with glyphosate at a rate of 0.84 kg ha^{-1} and then split in half by cultivation with a tandem disc in a split plot design to compare termination with herbicide only versus herbicide plus tillage. The main plot factor was prior N fertilization (N=4), with tillage being the subplot factor.

4.2.3 Sampling protocol and storage

Due to the large size of the plots, composite sampling was used to account for local variation within each plot. At each sampling time, four samples were taken from random locations within each plot and mixed together in a bucket, with a subsample taken from this mix. Soil samples from 0 to 15, 15 to 30, and 30 to 60 cm depth increments were taken at the beginning and end of the study for soil characterization and change over the course of the study. After the termination methods were employed, composite soil samples from the top 10 cm were taken weekly until freeze up, and then every two weeks for the following growing season (April – October). Soil samples were frozen at approximately -20 °C until all samples were collected for the season, after which they were air-dried, homogenized, and passed through a 2 mm dia. sieve.

4.2.4 Laboratory analysis

4.2.4.1 Soil characterization

Soil characterization measurements were done on samples from the 0 to 15, 15 to 30, and 30 to 60 cm depth increments and were completed by technical staff in the Soil Science Department at the University of Saskatchewan. Soil moisture content was determined for all soil samples by weighing approximately 25 g of air dry soil from each sample and drying in an oven at 105 °C for 24 h to reach a stable oven-dry weight. Once the samples returned to room

temperature they were re-weighed, and soil moisture content was determined (Eq. 3.1). Soil moisture content values were used to provide an oven dry weight equivalent for total, light fraction, and water extractable organic carbon measurements.

$$\text{Soil moisture content} = \frac{\text{air dry soil weight (g)-oven dry soil weight (g)}}{\text{oven dry soil weight (g)}} \quad \text{.....(Eq. 4.1)}$$

Soil inorganic N (NO_3^--N and NH_4^+-N, µg g^{-1}) was extracted using a 2*M* KCl solution (Keeney and Nelson, 1982). For each sample, 5.00 to 5.09 g of dried, ground soil and 50 mL of 2*M* KCl solution were placed in a 250 mL HDPE bottle. The bottles were shaken on a rotary shaker (G10 Gyrotory Shaker, New Brunswick Scientific Co., Edison, NJ, USA) for 1 h at 142 rpm and filtered through VWR 454 grade filter paper (VWR International LLC, Radnor, PA, USA) into 7-dram vials. Concentrations of soil inorganic N in the filtrate were analyzed colorimetrically using the Technicon AutoAnalyzer (Technicon Industrial Systems, Tarrytown, NY).

The modified Kelowna extraction (Qian et al., 1994) was used to determine soil inorganic PO_4^{3-}-P and K (available P and K, µg g^{-1}). For each sample, 3.00 to 3.09 g of dried, ground soil was weighed into 250 mL HDPE bottles, and 30 mL Kelowna solution (0.015*M* ammonium fluoride, 0.25*M* ammonium acetate, and 0.25*M* acetic acid) was added. The bottles were shaken on a rotary shaker for 5 min at 142 rpm and filtered through VWR 454 grade filter paper into 7-dram vials. Phosphate P concentration of the filtrate was determined colorimetrically using the Technicon AutoAnalyzer. Potassium concentration of the filtrate was determined using atomic emission (Varian Spectra 220 AAS; Varian Inc., Palo Alto, CA, USA).

4.2.4.2 Total organic carbon

Total organic C was measured by dry combustion and subsequent IR detection using the LECO C632 C analyzer (LECO Corporation, St. Joseph, MI, USA). Soil samples from the 0 to 15 and 15 to 30 cm depth increments were mechanically ground in a ball mill until they passed a 250 µm sieve (#60). Two hundred mg of each sample were weighed into ceramic crucibles, wetted with 1 mL of deionized water, and fumigated with 12*M* HCl for 48 h in sealed desiccators to remove carbonates. After fumigation, samples were placed in a fume hood for 48 h and then an oven for 24 h to remove moisture and residual HCl. The TOC was determined by combusting

the fumigated samples in the C632 at 1100 °C for 120 seconds. Mineral soil standards were used to calibrate the high and low IR detector cells.

4.2.4.3 Light fraction organic carbon

Light fraction organic carbon was determined by density fractionation using a sodium iodide solution with a specific gravity of 1.7 g cm^{-3} (Gregorich and Beare, 2008). Air dried soil samples were sieved to 2 mm, with residues retained on the sieves discarded. Forty g of sieved soil from each sample was placed in a plastic container with 80 mL of NaI solution. The containers were capped and shaken on a reciprocating shaker for 1 h. After shaking, the samples were washed from the containers into 250 mL glass beakers using the NaI solution, covered to minimize evaporation and air disturbance, and allowed to settle for 48 h. At the end of the settling period, the floating LF material was aspirated and washed with 75 mL 0.01*M* $CaCl_2$ followed by 75 mL distilled water. The washed LF material was dried for 24 h at 60 °C, weighed, combined based on treatment and sampling period, ground to pass a 250 µm sieve, and analyzed for C content on the C632. The amount of soil C contained in the light fraction is calculated as follows:

$$\text{LFOC (mg kg}^{-1}) = \frac{\text{fraction}_{dw} \text{ x LF C}}{\text{ODS wt}} \quad \text{.....(eq. 4.2)}$$

Where fraction_{dw} is the dry weight of the LF organic matter, LF C is the concentration of C in the LF sample, and ODS wt is the oven dry equivalent weight of each soil sample.

4.2.4.4 Water extractable organic carbon

Carbon in soil solution was measured using the water extraction method outlined by Chantigny et al. (2008). Ten g of sieved soil (dry mass basis) was placed in a glass test tube and gently stirred with 20 mL of 5 m*M* $CaCl_2$ for 1 min. The slurry was filtered through a 0.4 µm polycarbonate filter under a low vacuum and the filtrate was analyzed immediately after extraction by combustion and NDIR detection using a Shimadzu TOC-V_{CPN} (Shimadzu Corporation, Japan).

4.2.4.5 Microbial biomass carbon

Microbial biomass carbon was estimated using the fumigation-extraction method outlined by Voroney et al. (2008). Roots and other plant residues were manually removed from each

sieved soil sample, and the sieved soil samples were then incubated at 45% field capacity for approximately one week before beginning the procedure to permit soil metabolism and moisture to stabilize.

Field capacity was determined by saturating a column of soil with water and allowing it to drain for 48 h. One hundred g of soil was weighed into a cylindrical plastic container with a perforated bottom. The soil was saturated with water and a cap was placed over the open end of the container to prevent water loss through evaporation. The containers stood vertically on a wire rack and were allowed to drain for 48 h. After the drainage period, the soil sample was weighed, dried in an oven at 105 °C for 24 h, and then re-weighed to determine the amount of water lost. The difference in mass between the drained soil sample and oven dry soil sample was used as the amount needed for 100% field capacity.

$$100\%\ \text{field capacity} = \frac{\text{drained soil sample (g)} - \text{dry soil sample (g)}}{\text{dry soil sample (g)}} \quad \text{.....(Eq. 4.3)}$$

After the incubation period, three 15 g portions of soil from each sample were oven dried at 105 °C for 24h to determine the water content of each sample, and six 30 g portions of soil from each sample were put into 100 mL glass bottles for the fumigation experiment. Three of the 30 g portions of soil from each sample were extracted immediately by adding 0.5 *M* K_2SO_4 at a ratio of 1:2 (oven dry soil (g): extractant volume (mL)), shaking on a rotary shaker for 1h, and then filtering the soil suspension through Whatman GF 934-AH filter paper. The other three 30g portions from each sample were fumigated with $CHCl_3$ for 24h and then extracted using the same method as the unfumigated samples. Extracts were frozen until ready for analysis. Carbon content of the extracts was measured by combustion and NDIR detection using a Shimadzu TOC-V_{CPN} (Shimadzu Corporation, Japan). Microbial biomass C concentrations were calculated according to Voroney et al. (2008) using a k_{EC} value of 0.35.

4.2.5 Statistical analyses

Statistical analysis was done using the MIXED procedure in SAS (version 9.4; SAS Institute Inc., Cary, NC, USA). The data were analyzed as a split block randomized complete block design, with block being random effects and tillage and fertilization history being a fixed effect. PROC UNIVARIATE was used to determine if the data, block effect, and residuals were normally distributed. The Folded Form F statistic was used to determine if variances were equal.

In cases where the data was not normally distributed, log transformation was used to achieve normality. Tukey's Honestly Significant Difference test with an α level of 0.05 was used to compare multiple treatment means.

4.3 Results

4.3.1 Soil nutrients

The concentrations of the soil available macronutrients were not significantly different among treatments at either Carrot River site for any treatment combination at the beginning and end of the study (Tables 4.1 and 4.2). The soils at CR1 site experienced a slight decrease in all four available nutrient concentrations from beginning to end, reflecting nutrient removal with harvested forage stand. The soils at CR2 also tended to decrease in nutrient concentrations over the course of the study with the exception of a slight increase in P concentrations. While the increase in P concentration was not significant for any treatment combination, the effect, although small was significant ($p<0.05$) when comparing 0N plots to 100N plots, having concentrations of 4.28 and 3.42 mg kg^{-1} respectively. Reduced soil available P with N fertilization might be the consequence of greater yield and crop removal in harvest in the N fertilized treatment over the two years.

Table 4.1 Mean concentration of major soil available macro nutrients measured in the top 15 cm of soil at the two Carrot River sites (CR1 and CR2) at the beginning of the study (August, 2013). Means (n=8) within a column followed by different letters denote significant differences ($p<0.05$). Tukey's HSD was used to compare treatment means. Ammonium and NO_3^- were extracted by 2*M* KCl solution and available P and K by modified Kelowna solution. No significant differences were detected between any treatment at either site.

Treatment	CR1				CR2			
	NH_4^+-N	NO_3^--N	PO_4^{3-}-P	K	NH_4^+-N	NO_3^--N	PO_4^{3-}-P	K
	mg kg^{-1}							
100N	4.64	5.31	11.9	135.3	4.52	10.0	2.79	126.7
0N	4.62	5.73	11.3	137.4	5.20	7.62	3.59	120.2

Table 4.2 Mean concentration of major soil nutrients measured in the top 15 cm of soil at the two Carrot River sites (CR1 and CR2) at the end of the study (October, 2014). Means (n=4) within a column followed by different letters denote significant differences (p<0.05). Tukey's HSD was used to compare treatment means. Ammonium and NO_3^- were extracted by 2*M* KCl solution and available P and K by modified Kelowna solution. No significant differences were detected between any treatment at either site.

Treatment	CR1				CR2			
	NH_4^+-N	NO_3^--N	PO_4^{3-}-P	K	NH_4^+-N	NO_3^--N	PO_4^{3-}-P	K
	---------- mg kg^{-1} ----------							
Tilled 100N	4.13	4.10	10.05	97.0	4.79	7.74	3.31	94.44
Not Tilled 100N	4.09	4.42	8.70	94.1	4.97	7.26	3.54	88.38
Tilled 0N	4.01	5.07	10.42	108.0	4.53	6.30	4.54	88.51
Not Tilled 0N	4.31	3.86	9.32	87.5	4.48	6.50	4.02	81.58

4.3.2 Light fraction organic carbon

The initial LFOC concentrations at CR1 were significantly higher in the 100N plots versus the 0N plots (Table 4.3). The mean LFOC concentrations at CR2 site were also higher in the 100N plots but the difference was not significant. The higher productivity of the fertilized forage stand explains this increase. At the end of the study, after stand termination and 2 years after N fertilization treatments ceased (Table 4.4), the LFOC concentrations increased in every treatment. This is attributed to the breakdown of the old root mass after the removal of the forage stand. The differences among treatments were not significant, but the tilled plots tended to have higher LFOC concentrations. A history of N fertilization appeared to negatively influence LFOC concentrations over the study, suggesting that the increased nutrient availability increased LFOC decomposition.

Table 4.3 Mean concentration of LFOC in the top 15 cm of soil measured at the beginning of the study (August, 2013). Means (n=8) within a column followed by different letters denote significant differences (p<0.05). Tukey's HSD was used to compare treatment means.

Treatment	CR1	CR2
	------mg C kg^{-1}------	
100N	558a	618a
0N	472b	543a

Table 4.4 Mean concentration of LFOC in the top 15 cm of soil measured at the end of the study (October, 2014). Means (n=4) within a column followed by different letters denote significant differences (p<0.05). There were no significant differences between treatments at either site. Tukey's HSD was used to compare treatment means.

Treatment	CR1	CR2
	-------- mg C kg^{-1} --------	
Tilled 100N	663	1085
Not Tilled 100N	609	1112
Tilled 0N	704	1245
Not Tilled 0N	682	1048

In all plots, there was an increase in LFOC concentrations following stand termination in the period from fall 2013 (T1) to spring 2014 (T2) (Tables 4.5 and 4.6). At the moister CR1 site, the average concentrations at the end of the study (T7) all tended to be lower than immediately after termination (T1). Conversely, the drier CR2 site likely had slower decomposition rates, and tended to have higher LFOC concentrations in fall of 2014 at the end of the study (T7) than immediately after termination (T1). There were no significant differences among tillage/fertilization treatments at either site at any of the three sample times. At the CR2 site, the tilled plots had significantly higher (p<0.05) LFOC concentrations in spring of 2014 (T2) than the non-tilled plots, with 1729 and 1365 mg kg^{-1} respectively (Table 4.7). At the end of the season (T7), the differences due to tillage disappeared.

Table 4.5 Mean concentration of LFOC in the top 10 cm of soil measured over time after forage stand termination at CR1 site. Samples were collected 1 wk following termination (T1, October 18, 2013), immediately after spring thaw the following year (T2, May 12, 2014), and after crop harvest at the end of the 2014 season (T3, September 29, 2014). Means (n=4) within a row followed by different letters denote significant differences (p<0.05). There was no significant difference between any treatment for any sampling time. Tukey's HSD was used to compare treatment means.

Sample time	Tilled 100N	Not Tilled 100N	Tilled 0N	Not Tilled 0N
	---------- mg C kg^{-1} ----------			
T1 18 Oct. 2013	884	977	984	999
T2 12 May 2014	1412	1476	1375	1062
T7 29 Sept. 2014	768	712	742	825

Table 4.6 Mean concentration of LFOC in the top 10 cm of soil measured over time after forage stand termination at CR2 site. Samples were collected 1 wk following termination (T1, October 18, 2013), immediately after spring thaw the following year (T2, May 12, 2014), and after crop harvest at the end of the 2014 season (T7, September 29, 2014). Means (n=4) within a row followed by different letters denote significant differences (p<0.05). There were no significant differences between any treatment at any sampling time. Tukey's HSD was used to compare treatment means.

Sample time	Tilled 100N	Not Tilled 100N	Tilled 0N	Not Tilled 0N
	---------- mg C kg^{-1} ----------			
T1 18 Oct. 2013	1012	927	1147	1102
T2 12 May 2014	1696	1325	1763	1405
T7 29 Sept. 2014	1238	1234	1299	1254

Table 4.7 Mean concentration of LFOC in the top 10 cm of soil measured over time after forage stand termination at CR2 site. Samples were collected 1 wk following termination (T1, October 18, 2013), immediately after spring thaw the following year (T2, May 12, 2014), and after crop harvest at the end of the 2014 season (T7, September 29, 2014). Means (n=8) within a row followed by different letters denote significant differences (p<0.05). Tukey's HSD was used to compare treatment means.

Sample time	Tilled	Not Tilled
	------ mg C kg^{-1} ------	
T1 18 Oct. 2013	1079a	1014a
T2 12 May 2014	1729a	1365b
T7 29 Sept. 2014	1268a	1244a

4.3.3 Water extractable organic carbon

At the CR2 site, N fertilization for two years resulted in significantly higher WEOC in the surface soil than the unfertilized treatment (Table 4.8). At CR1, there was no influence. The WEOC concentrations at CR1 site showed no significant differences among any of the treatments in the top 15 cm of soil at the beginning and end (Table 4.9) of the study. The WEOC concentrations at CR2 were significantly higher in the 100N treatment compared to the 0N treatment at the beginning of the study in August, 2013, but the differences disappeared by the final sampling period in October, 2014. The WEOC declined in all treatments over the course of the study. When examining the overall reduction in WEOC from August, 2013 to October, 2014 (Fig 4.1), fertilization history had the largest effect. The reduction in WEOC in 100N plots was up to 2.5 times that of the 0N plots. The additional N supply may have fuelled microbial activity and utilization of the water soluble organic C as substrate.

Table 4.8 Mean concentration of WEOC in the top 15 cm of soil measured at the beginning of the study (August, 2013). Means (n=8) within a column followed by different letters denotes significant differences (p<0.05). Tukey's HSD was used to compare treatment means.

Treatment	CR1	CR2
	------ mg C kg^{-1} ------	
100N	105.3a	177.3a
0N	108.1a	137.5b

Table 4.9 Mean concentration of WEOC in the top 15 cm of soil measured at the end of the study (October, 2014). Means (n=4) within a row followed by different letters denotes significant differences (p<0.05). Tukey's HSD was used to compare treatment means. There were no significant differences between any treatment at either site.

Treatment	CR1	CR2
	--- mg C kg^{-1} ---	
Tilled 100N	77.7	123.8
Not Tilled 100N	83.6	128.7
Tilled 0N	86.6	117.7
Not Tilled 0N	84.9	116.0

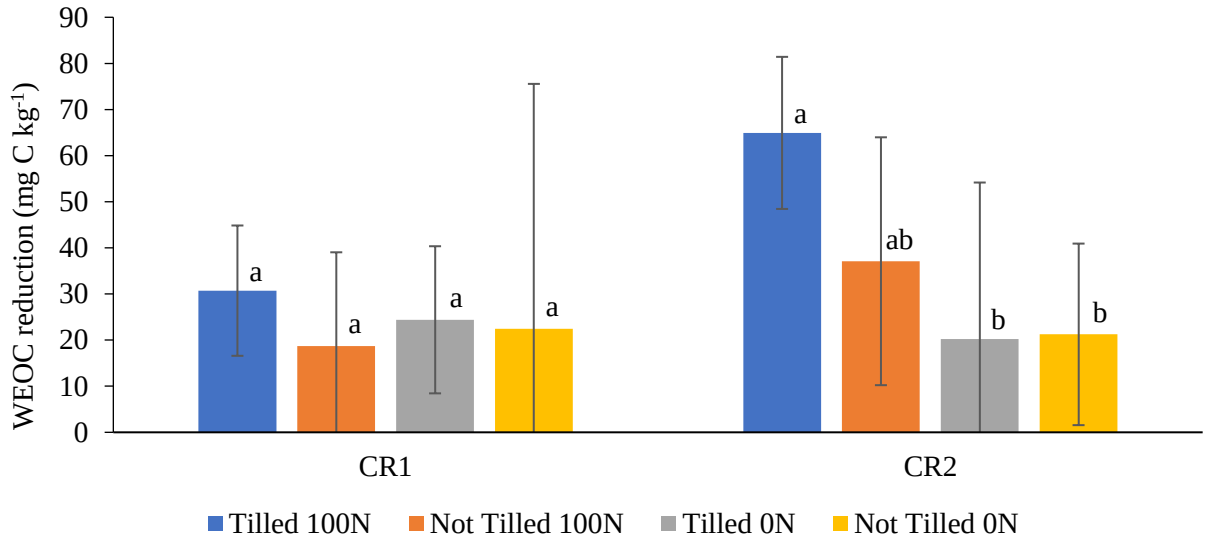

Figure 4.1 Mean reduction of WEOC concentration in the top 15 cm of soil from August, 2013 to October, 2014. Letters denote significant differences (p<0.05) between means (n=4) within each site (CR1 and CR2). Error bars represent one standard deviation. Tukey's HSD was used to compare treatment means.

The general pattern followed by the WEOC concentrations in the top 10 cm at both sites over time (Tables 4.10 and 4.11) was a peak immediately following stand termination (T1), decreasing to often the lowest values immediately after spring thaw (T2), and then tended to increase by the end of the 2014 season (T7). At CR1, there were significant treatment differences one week after stand termination (T1) with the non-tilled plots having higher WEOC concentrations than tilled plots. After spring thaw, treatment differences disappear until the beginning of August (T5) where the tilled 100N plots had significantly lower WEOC concentrations than the other 3 treatments, but the effect was small when compared to the ranges

at other sampling times. The CR2 site followed a similar pattern to CR1 but there were no significant differences among any of the treatments. Interestingly, both sites had significantly higher WEOC concentrations in the non-tilled plots at the beginning of August (T5).

Table 4.10 Mean concentration of WEOC in the top 10 cm of soil measured after forage stand termination at CR1 site. Samples were collected 1 wk following termination (T1, October 18, 2013), immediately after spring thaw the following year (T2, May 12, 2014), then every 4 wks until the end of the 2014 season (T3-T7, September 29, 2014). Means (n=4) within a row followed by different letters denote significant differences ($p<0.05$). Tukey's HSD was used to compare treatment means.

Sample time	Tilled 100N	Not Tilled 100N	Tilled 0N	Not Tilled 0N
	---------- mg C kg^{-1} ----------			
T1 18 Oct. 2013	101.8c	113.7ab	102.3bc	117.7a
T2 12 May 2014	77.2a	74.5a	76.5a	82.6a
T3 9 June 2014	78.9a	77.6a	81.4a	81.3a
T4 7 July 2014	84.2a	80.2a	73.2a	80.4a
T5 5 Aug. 2014	77.7b	88.9a	81.9b	85.1ab
T6 2 Sept. 2014	82.4a	89.8a	80.7a	83.7a
T7 29 Sept. 2014	97.6a	101.6a	96.0a	107.3a

Table 4.11 Mean concentration of WEOC in the top 10 cm of soil measured after forage stand termination at CR2 site. Samples were collected 1 wk following termination (T1, October 18, 2013), immediately after spring thaw the following year (T2, May 12, 2014), then every 4 wks until the end of the 2014 season (T3-T7, September 29, 2014). Means (n=4) within a row followed by different letters denote significant differences (p<0.05). There were no significant differences between treatments at any sampling time. Tukey's HSD was used to compare treatment means.

Sample time	Tilled 100N	Not Tilled 100N	Tilled 0N	Not Tilled 0N
	---------- mg C kg^{-1} ----------			
T1 18 Oct. 2013	164.5	169.2	141.8	153.5
T2 12 May 2014	92.2	109.1	96.2	97.1
T3 9 June 2014	129.0	134.3	120.9	118.3
T4 7 July 2014	120.5	118.4	113.8	110.2
T5 5 Aug. 2014	126.2	132.8	96.4	120.9
T6 2 Sept. 2014	119.6	130.9	114.6	116.1
T7 29 Sept. 2014	141.8	151.8	125.8	143.1

4.3.4 Microbial biomass carbon

Microbial biomass C measured in the top 15 cm at the CR1 site showed no significant differences among any treatments but tended to decrease over the course of the study (Tables 4.12 and 4.13). At the CR2 site, the 100N treatment had significantly higher MBC concentrations than the 0N plots in August 2013 at the beginning of the study, but the difference was no longer significant at the end of the study in October 2014. The two years of N fertilization appear to have stimulated microbial growth in the surface soil of the CR2 site. One year later, the non-tilled 100N still has mean microbial biomass that is ~ 75 mg C kg^{-1} higher than the 0N non-tilled treatment (Table 4.12) but it is not significantly different. In general, past fertilization with N tended to increase MBC.

Table 4.12 Mean concentrations of microbial biomass carbon (MBC) in the top 15 cm of soil measured at the beginning of the study (August, 2013). Means (n=8) within a column followed by different letters denotes significant differences (p<0.05). Tukey's HSD was used to compare treatment means.

Treatment	CR1	CR2
	----- mg C kg^{-1} -----	
100N	107.8a	215.1a
0N	127.3a	98.6b

Table 4.13 Mean concentrations of MBC in the top 15 cm of soil measured at the end of the study (October, 2014). Means (n=4) within a row followed by different letters denotes significant differences (p<0.05). There were no significant differences between treatments at either site. Tukey's HSD was used to compare treatment means.

Treatment	CR1	CR2
	------ mg C kg^{-1} ------	
Tilled 100N	112.8	121.5
Not Tilled 100N	104.6	148.7
Tilled 0N	105.7	116.6
Not Tilled 0N	113.6	73.75

Closely following the LFOC concentrations, the MBC concentrations measured over time after termination of the stands at CR1 (Table 4.14) and CR2 (Table 4.15) sites peaked after spring thaw (T2) likely due to increased moisture availability and an abundance of readily decomposable C produced by freeze-thaw cycles. There were no significant differences at either site.

Table 4.14 Mean concentrations of MBC in the top 10 cm of soil measured after forage stand termination at CR1 site. Samples were collected 1 wk following termination (T1, October 18, 2013), immediately after spring thaw the following year (T2, May 12, 2014), and after crop removal at the end of the 2014 season (T7, September 29, 2014). Means (n=4) within a row followed by different letters denote significant differences (p<0.05). There were no significant differences between treatments at any sample time. Tukey's HSD was used to compare treatment means.

Sample time	Tilled 100N	Not Tilled 100N	Tilled 0N	Not Tilled 0N
	----------mg kg^{-1}----------			
T1 18 Oct. 2013	56.6	95.2	85.9	89.2
T2 12 May 2014	158.6	162.3	141.2	218.8
T7 29 Sept. 2014	40.0	89.8	84.1	91.0

Table 4.15 Mean concentrations of MBC in the top 10 cm of soil measured after forage stand termination at CR2 site. Samples were collected 1 wk following termination (T1, October 18, 2013), immediately after spring thaw the following year (T2, May 12, 2014), and after crop harvest at the end of the 2014 season (T7, September 29, 2014). Means (n=4) within a row followed by different letters denote significant differences (p<0.05). There were no significant differences between treatments at any sample time. Tukey's HSD was used to compare treatment means.

Sample time	Tilled 100N	Not Tilled 100N	Tilled 0N	Not Tilled 0N
	---------- mg kg^{-1} ----------			
T1 18 Oct. 2013	38.2	72.7	99.6	47.7
T2 12 May 2014	209.7	206.0	173.1	209.3
T7 29 Sept. 2014	123.2	108.3	102.4	89.6

4.3.5 Total soil organic carbon

There were no significant differences in TOC concentrations among treatments at either site at the beginning before the tillage treatments were imposed (Table 4.16) and at the end among all tillage/fertilization treatments (Table 4.17). When examining the initial TOC concentration, there is a marked difference between the two CR sites, with CR2 having approximately 47-116% higher total SOC concentrations than CR1. This was also evident in all three of the analysed C pools, where CR2 tended to have higher concentrations than CR1. At the end of the study, the C concentrations in all treatments at a site were similar. Termination with

tillage did not appear to result in measurable difference in TOC concentration compared to no tillage termination.

Table 4.16 Mean concentrations of total soil organic carbon (SOC) in the top 15 cm measured at the beginning of the study (August, 2013). Means (n=8) within a row followed by different letters denote significant differences (p<0.05). There were no significant differences between treatments at either site. Tukey's HSD was used to compare treatment means.

Site	CR1	CR2
	-------- g kg^{-1} --------	
100N	23.27	50.37
0N	26.12	38.45

Table 4.17 Mean concentrations of TOC in the top 15 cm measured at the end of the study (October, 2014). Means (n=4) within a row followed by different letters denote significant differences (p<0.05). There were no significant differences between treatments at either site. Tukey's HSD was used to compare treatment means.

Treatment	CR1	CR2
	----- mg C kg^{-1} -----	
Tilled 100N	19.39	41.63
Not Tilled 100N	19.40	38.48
Tilled 0N	22.70	40.49
Not Tilled 0N	20.26	40.95

4.4 Discussion

4.4.1 Soil nutrients

The initial nutrient concentrations at both CR sites were not significantly affected by N fertilizer applications made for the past two years. The major difference was evident in available macronutrient concentrations between the two sites, with CR1 site having 4 times more available P and just over half the NO_3^- concentration (Table 4.1). Higher N availability at CR2 site may lead to more immobilization of P in biomass, as N increases forage dry mass production and can lead to a decrease in soil P concentrations (Dillard et al., 2015). At both CR sites, a negative nutrient balance due to crop removal without fertilization in the season following stand termination, explains the decrease in available nutrient concentrations.

4.4.2 Light fraction organic carbon

There were no significant differences among treatments at either site one week after stand termination (Tables 4.5, 4.6). The 100N plots at both sites tended to have lower LFOC concentrations than the 0N plots, suggesting that initial decomposition may be accelerated with fertilizer N having been applied in the past. The differences in LFOC concentrations due to tillage were more pronounced at the drier CR2 site, where tilled plots had up to 27% higher LFOC concentrations than the non-tilled plots. By the end of the season, the difference in LFOC concentration among treatments was not significant, suggesting that the increased amount of LFOC found initially in the tilled plots was largely decomposed within a year of stand termination.

Nitrogen fertilization would increase LFOC concentrations through increased biomass production and rhizodeposition. This effect is evident when comparing the 100N plots to the 0N plots at the beginning of the study (Table 4.3), where the 100N plots had 13-18% higher LFOC concentrations compared to the 0N plots. A study by Malhi et al. (2003) in Crossfield, Alberta showed that LFOC concentrations increased with increasing N fertilizer rates to a maximum at 224 kg N ha^{-1} yr^{-1} in the top 5 cm and at the 336 kg N ha^{-1} yr^{-1} rate in the 5 to 15 cm depth range. The average annual increase in LFOC concentrations due to N fertilization in the study by Malhi et al. (2003) is over 10 times higher than the annual increase at either Carrot River site, suggesting that LFOC concentrations build up faster over time as the root mass continues to grow. The differences in initial LFOC concentrations were not significant at CR2 but may become significant given another year of forage growth and N fertilizer addition.

4.4.3 Water extractable organic carbon

Termination by tillage tended to have a negative effect on WEOC concentrations over the following year at both sites. The WEOC concentrations at both CR sites were highest after termination (Tables 4.10, 4.11), and were at their lowest immediately after spring thaw, decreasing by 32-44%. A review by Chantigny (2003) on the influence of land use and management practices on dissolved organic matter in soils suggests that WEOC concentration decreases after the removal of grass species due to a gradual depletion in soil organic matter. The effect of tillage on WEOC concentrations is less clear, as the reported effects in the literature range from negative to positive (Chantigny, 2003; Zhang et al., 2011). The 100N treatment plots

at CR2 had significantly higher WEOC concentrations compared to the 0N plots, being on average 29% higher (Table 4.8). The seasonally waterlogged CR1 site did not show any significant differences due to fertilization history.

After the following season's crop was planted, WEOC in the top 10 cm at both sites gradually increased over the course of the season, recovering to 86-96% of the previous years concentrations (Tables 4.10, 4.11). The recovery of WEOC in the top 15 cm (Tables 4.8, 4.9) was much lower than in the top 10 cm, ranging from 70-86%, and was lowest in the 100N plots. This suggests that annual N fertilization can increase microbial metabolism in the soil for one or more years after addition, and this increased metabolism and decomposition is removing C from depth over time. When comparing the average reduction in WEOC in the top 15 cm over the course of the study (Fig 4.1), a history of N fertilization followed by tillage resulted in the highest reductions in WEOC, suggesting a greater degree of substrate consumption in this treatment.

4.4.4 Microbial biomass carbon

After the stand was terminated, the tilled plots tended to have lower MBC concentration than the non-tilled plots for the duration of the study. This is likely a move towards a new equilibrium, as uncultivated soils typically support more microbial biomass than cultivated soils (Helgason et al., 2009). Temporal variation in MBC concentrations (Tables 4.15, 4.16) mirrored that of the LFOC concentrations (Tables 4.5, 4.6) and were opposite to WEOC concentrations (Tables 4.11, 4.12), suggesting that readily available WEOC is used as a C and energy source when decomposing LFOC. As the amount of LFOC declined over the 2014 season, so too did the MBC, suggesting that WEOC is an immediate source while LFOC is short-term substrate gradually consumed over months by the soil microbes.

Similar to LFOC and WEOC concentrations, the initial MBC concentrations were significantly higher in the 100N plots compared to the 0N plots (Table 4.13). Many studies have reported positive response of microbial biomass to repeated fertilizer additions across varying soil types, attributing this to the higher N availability supporting a larger microbial community (Li et al., 2005; Lupwayi et al., 2010; Zhang et al., 2015).

4.4.5 Total organic carbon

Mean TOC concentrations generally decreased over the course of the study as a new equilibrium was reached, but treatment effects of N fertilization history and termination method were not significant; not surprising given the short period of time over which the effect of the treatment was evaluated.

4.5 Conclusions

The research described in this chapter examined the response of several soil C fractions in forage stands at two locations in northeastern Saskatchewan to past N fertilization history and termination method of tillage versus no-till. The findings suggest that in these poorly drained, high clay soils from the Gray-Black soil climatic zone, grass stand termination including tillage may not negatively affect soil C concentrations.

Nutrient concentrations in the soil do not appear to be affected by termination method, as the differences among treatments were small and not significant. The effects of each treatment on soil N concentrations tended to be greater in the drier CR2 site, whereas effects on P and K availability were larger in the CR 1 site where there was greater frequency of waterlogging.

Light fraction organic carbon concentrations were significantly influenced by termination method, as termination with tillage resulted in a larger spike in LFOC concentration immediately after spring thaw. The LFOC is a transitory component derived from litter and root mass in the early stages of decomposition and, as an “active” fraction, represents a relatively large component of the total SOC compared to water extractable and microbial biomass C pools. Creation of LFOC from the terminated crop may extend to multiple years as the root system continues to decay. Past N fertilization significantly increased LFOC concentrations, but the increase did not translate to higher rates of LFOC degradation. Similarly, initial WEOC concentrations were significantly increased in response to prior N fertilizer application. However, the increased amount of WEOC in the 100N plots was consumed over the course of the study, as shown by the lack of differences among WEOC in treatments at the end of the study. Termination by tillage did not have any significant effect on WEOC concentrations but could have been missed since the WEOC is a small but highly dynamic fraction, likely changing over days in response to differing conditions.

Microbial biomass was influenced by both past N fertilization and termination method. The N fertilized plots likely stimulated formation of MBC through the growth of the forage stand and maintained this larger amount of biomass. Stand termination by tillage tended to reduce MBC concentrations in the soil, which in turn may slow the breakdown of the various C fractions in the soil.

When examining total SOC change associated with the land use change from grass forage to an annual crop of hemp, the termination by tillage did not accelerate the decline in SOC compared to no-till. Therefore, while tillage is frequently reported to increase the rate of decomposition and decrease SOC (Larney et al., 1997; Abdalla et al., 2013), one tillage operation associated with grass stand termination appeared to have little effect versus termination with herbicide only.

With up to a 20% decrease in total SOC in the first year after stand termination but with no significant effect of treatment, future studies would benefit from more years of monitoring. This would likely capture the new equilibrium point for the various C fractions and would allow for better estimates on the effects of each treatment. Furthermore, measuring litter and heavy fraction organic C concentrations would add vital information as to where the C is cycling through the soil profile.

5.0 SYNTHESIS AND CONCLUSIONS

5.1 Overview

The studies described in the previous two chapters were conducted to examine the influence that forage stand termination method and N fertilization history has on soil greenhouse gas emissions, soil C fractions, and soil nutrient mobility. This research is important for identification of beneficial management practices (BMPs) for the forage industry that will minimize negative environmental impacts and maximize benefits to producers.

A laboratory incubation was conducted using intact soil cores collected from bromegrass and timothy small-plot replicated trials in north-eastern Saskatchewan. The influence of N fertilization history (+N or -N) and termination method (tillage or herbicide) on greenhouse gas emissions, nutrient supply rates, and nutrient leaching is described in Chapter 3. Termination by tillage reduced greenhouse gas emissions and nutrient leaching in the short-term (first year), which may be explained by the poorly drained, high clay content of the soils. The seasonally waterlogged Carrot River field site (CR1 site) showed the largest reduction in greenhouse gas emissions, specifically N_2O emissions, in response to tillage where improved aeration effects would likely be most evident. Conversely, termination by tillage reduced available nutrient supply rates, possibly through enhanced microbial immobilization, which may increase fertilizer needs for a subsequent annual crop, although this effect would likely disappear later in the season after the initial increase in substrate and O_2.

As expected, prior applications of fertilizer N to the grass forage stands increased emissions of N_2O measured following stand termination compared to treatments without N fertilizer added for the previous two years. The N supply rates tended to be higher in the fertilized plots, but N fertilization also tended to decrease P supply rates, likely through a combination of enhanced P immobilization and greater P removal in the fertilized biomass. Nitrogen leaching rates tended to be higher in the N fertilized plots but the effect was not significant. The findings from this study suggest that stand termination by tillage in these wet, high clay content soils may be effective in reducing greenhouse gas emissions and nutrient

leaching in the following year, but the longer-term consequences of this remains unknown.

The second goal of this thesis was to quantify changes in soil C amounts and forms in response to termination method. Previous work has shown that forage crops significantly increase C in the soil (Mensah et al., 2003). Further to this, N fertilization greatly increases C storage by forages, especially in grasses like bromegrass (Malhi et al., 2004; Lkhagvasuren et al., 2011). The field study covered in Chapter 4 of this thesis examined the change in several soil C fractions and soil nutrients to forage stand termination method in the year following termination. The differences in soil nutrient concentrations between the termination treatments were small and not significant. The drier Carrot River site (CR2 site) tended to exhibit larger treatment effects on N concentrations, whereas the effects on P and K concentrations were greater at the seasonally waterlogged CR1 site. Termination by tillage caused a significant spike in LFOC concentrations immediately after spring thaw which disappeared over the course of the growing season. Concentrations of LFOC were significantly higher in the N fertilized plots, but this did not translate to higher degradation rates. Treatments with N fertilization history also had significantly higher WEOC concentrations in the soil, but the WEOC was consumed over the course of the study as evidenced by the lack of differences between treatments at the end of the study. Termination treatment effects on WEOC concentrations were not observed, explained by the highly dynamic nature of WEOC and which may have been influenced only in the first days following termination. Both N fertilization history and termination method influenced MBC concentrations. Improved growth of the forage stand in the 100N plots would likely stimulate microbial activity and MBC formation, which was maintained after stand termination. It was anticipated that tillage would increase microbial biomass and SOC decomposition due to enhanced aeration. Termination by tillage, however, tended to reduce MBC concentrations, suggesting concomitant reduced decomposition of the various soil C fractions. When examining the overall change in total SOC, termination by tillage did not cause an apparent increase in SOC decomposition versus non-tilled treatments as evidenced by similar SOC levels at the end of the season. This suggests that tillage operations on these wet, high-clay soils has minimal effects on SOC storage over the short-term compared to termination by herbicide alone.

5.2 Synthesis and Recommendations

According to the Saskatchewan Forage Council, the BMP for terminating grass forage stands recommends a combination of herbicide followed by tillage (Sask Forage Council, 1998a, b). This recommendation is based on the resilience of bromegrass to termination, with the aim of reducing the energy requirement of breaking the stand up solely relying on tillage operations. Control can be further enhanced the following year by the application of a graminicide. The results of this thesis work provide further support for these practices in the context of greenhouse gas emission effects, specifically that tillage can be used to effectively terminate a forage stand without significantly decreasing the stored SOC. In poorly drained, wet soils such as the CR1 site, tillage may provide some benefit in reducing N_2O emissions due to improved aeration. However, this study also revealed that N fertilization history will influence N_2O emissions, with higher emissions evident from the terminated grass stands with a history of N fertilizer added in previous years. Despite lower emissions from stands without N fertilizer added, productivity of the grass stand in both biomass and grass seed yield is anticipated to suffer from insufficient supplies of soil N.

It is noteworthy that the N_2O emissions measured in this study were relatively low compared to those determined in the previous 2 years when the N fertilizer applications were made in the field, especially at the Carrot River site. Field N_2O emission measurements made by Nils Yannikos (Yannikos, 2016) on these plots in the spring of 2013 revealed daily N_2O fluxes that were as much as 11 and 39 times higher than the highest measured fluxes in this study at CR1 and CR2, respectively. Ambient air temperature on those field days was also much lower than the temperature used in the laboratory incubation, ranging from 5 – 15 °C compared to 25 °C in the laboratory. The greater N_2O emissions observed by Yannikos (2016) in the years in which the N fertilizer applications were made versus following years is explained primarily by initial emissions arising from nitrification and denitrification of the applied fertilizer N prior to plant uptake. At the beginning of the current study, ammonium and nitrate N levels that could contribute to N_2O production were relatively low as a result of crop and microbial utilization.

The results of these studies suggest that tillage can safely be used to terminate a forage stand in these clayey, poorly drained soils from the Gray-Black soil climatic zone. The GHG emission reduction from tillage tended to be more pronounced in the wetter areas, where the improved aeration from tillage may have been responsible for significantly decreased N_2O

emissions by reducing denitrification. At the wetter CR1 site, the range in CO_2 emissions between treatments was smaller than the range in CO_2 emissions at the CR2 site, while the opposite was true for N_2O emissions. This suggests that wetter areas benefit more from termination by tillage than drier areas, as there was no significant difference between CO_2 emissions while N_2O emissions are significantly reduced. The emissions findings in Chapter 3 are supported by the effects on the soil C pools examined in Chapter 4. Tillage tended to decrease MBC concentrations in the soil, consistent with lowered decomposition rates of SOC while the non-tilled plots maintained more MBC over the following season, which is consistent with the higher CO_2 emissions in the non-tilled plots. When examining the total SOC change at both sites, termination by tillage did not result in the expected decrease in SOC concentration compared to no-till. It appears that a single tillage operation to terminate a grass forage stand has limited effect compared to herbicide alone.

5.3 Future Research

While the findings of this thesis support the current BMP of including tillage in the termination strategy, particularly through its effect on reducing N_2O, these findings could change from a similar study with more frequent sampling. First, WEOC is an extremely mobile and dynamic fraction of total SOC. Some of the differences in WEOC concentrations likely disappeared before the first sampling time, as evidenced by the lack of significant differences between termination treatments at CR1 and only minor differences at CR2. Daily or hourly measurements made *in situ* immediately following termination may have increased the ability to capture termination method effects on WEOC. Secondly, it is likely that significant GHG emissions could occur immediately after spring thaw when the field was waterlogged and inaccessible, especially N_2O emissions. Field measurement units and automated chambers installed immediately after termination would be able to capture these unknown emissions which could represent a significant proportion of overall emissions.

This study would also have benefitted from examining other soil C pools and for a longer time period. Comparatively, LFOC is a large but also active component of total SOC. The tilled plots had a significant spike in LFOC concentrations immediately following spring thaw that disappeared by the end of the season. As this decrease in LFOC concentrations was not reflected in the CO_2 emissions, measuring the heavy fractions of organic C would help elucidate where the

C is cycling from in the soil. Furthermore, had this study continued for more than one year following termination, significant treatment differences may have become evident as the soil moves to a new equilibrium. Lastly, this thesis did not consider the GHG emissions from fuel used by equipment involved in tillage and herbicide termination. Equipment emissions would perhaps offset any reduction gained from tillage and merits further attention.

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